The Synthesis and Biological Evaluation of the Pyrrolidine Antibiotic Anisomycin

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February 2003

Declaration

This thesis is submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy at The University of Edinburgh. Unless otherwise stated the work described in this thesis is original and has not been submitted previously in whole or in part for any degree or other qualification at this, or any other university. In accordance with the regulations this thesis does not exceed 70,000 words in length.

Edward Michael Rosser

Acknowledgements

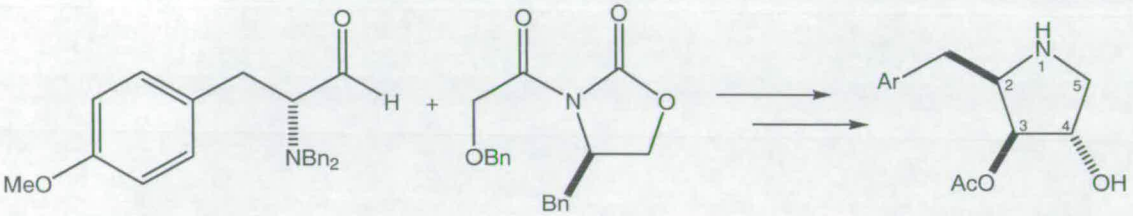
I would like to thank my supervisor, Dr. Alison Hulme, for her help, encouragement and advice throughout and for her thorough proofreading of this thesis.

I would also like to thank Professor Philip Cohen, Professor Rob Field, and Simon Morton at the Universities of Dundee and East Anglia for carrying out various biological assays. Thanks also go to Dr. David Reed, John Millar and Wesley Kerr for running numerous NMR spectra for me. Thanks to Alan Taylor and Harry Mckenzie for their mass spectrometry work and Dr. Simon Parsons for his X-ray diffraction work.

I would like to thank Dr Andrew March for proofreading this thesis, and for 'keeping it scotch'. Thanks also to Benny Benstead for keeping up morale during the cold winter of 2001/02, and for inventing corridor cricket. Thanks also to Mr Murphy, Kazza Curly, Sarah Baron, Kate Longden and Jenny Aird all of whom provided much needed hilarity. Finally, last but not least, a big thank you to Rachel for her love, support, and encouragement over the last 3 years, and most of all for her patience.

Abstract

This thesis documents the synthesis of the antibiotic anisomycin **10**, whose use as a tool for the activation of the mitogen-activated protein (MAP) JNK and p38 stress kinase pathways is well documented. The synthesis makes use of a highly efficient asymmetric boron mediated *syn* glycolate aldol reaction employing the tyrosine derived aldehyde **31** and acylated Evans' auxiliary **32**

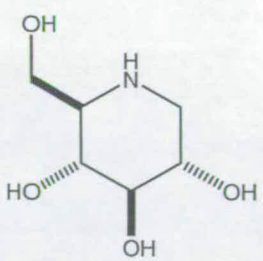


31 **32** **10**

The synthesis of the more lipophilic C(4) analogues **82** and **84** is also described and a SAR study of these and their synthetic precursors on the p38 and JNK kinase signalling pathways is presented.

82 **84**

An investigation into the asymmetric boron mediated glycolate aldol reaction employed in the synthesis of anisomycin is also reported. Finally studies into extending our methodology to incorporate six membered piperidine rings is discussed in our approach to the synthesis of the glycosidase inhibitor 1-deoxynojirimycin (DNJ) **164**.



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Chapter 1: Introduction

1.1 Anisomycin

Anisomycin was originally isolated from the fermentation broths of *Streptomyces griseolus* and *Sreptomyces roseochromogenes* by Stobin and Tanner in 1954.[1] Since its discovery it has also been isolated from *Streptomyces sp. SA3097*[2] and *No 638*[3] by two other groups in Japan. The gross structure of anisomycin was elucidated by Beereboom in 1965,[4] and its relative stereochemistry was established by NMR[5] and X-Ray analysis.[6] Wong[7] finally confirmed the absolute stereochemistry to be (2*R*,3*S*,4*S*) in 1968 by chemical correlation with L-tyrosine (**Figure 1**).

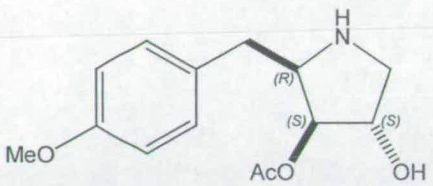

Figure 1 : Anisomycin

Anisomycin's activity as an antibiotic was first tested *in vitro* by Lynch.[8] It was found that *E. histolytica, T. vaginalis*, *T. foetus*, and *Candida albicans* were all inhibited by anisomycin, requiring concentrations of only 1 μg per ml of cell culture. Consequently, anisomycin was used in clinical trials for the treatment of both amoebic dysentery[9] and vaginitis caused by *Trichomonas vaginalis*.[10] However in the latter, the trials were prevented from progressing due to the uncertainty of success and the inconvenient method of application. Anisomycin was found to be inactive towards bacteria at realistic concentrations, with *Staphalococus aureus*, *Streptomyces faecalis*, mycobacteria and Gram-negative organisms all requiring more than 100 μg per ml of cell culture for inhibition.

Anisomycin's ability to effectively inhibit peptide bond formation on eukaryotic ribosomes, as well as its relatively low toxicity, has made it an invaluable tool in

molecular biology.[11] Anisomycin has been used to show that various processes occurring within the body are the result of protein synthesis. For example, the acquired tolerance to alcohol of laboratory rats was suppressed when an initial dose of alcohol was preceded by an injection of anisomycin, indicating the presence of a protein that provides tolerance to, and is stimulated by alcohol.[12]

In a similar study anisomycin was used to show that consolidated 'fear memories', when reactivated during retrieval, return to a labile state that requires *de novo* protein synthesis for reconsolidation.[13] It was found that when laboratory rats were treated with anisomycin shortly after 'fear memory' reactivation, they showed amnesia towards later tests. The same treatment with anisomycin 6 hours after the fear memory reactivation, as well as in the absence of any 'fear memory' reactivation, left the rats 'fear memory' intact.

Recently, anisomycin has been shown to display cytotoxicity towards human tumour cell lines *in vitro*[2,14] and to activate the mitogen-activated protein (MAP) JNK and p38 stress kinase pathways.[15]

1.2 Anisomycin as an Antibiotic

In contrast to many antibacterial antibiotics, anisomycin acts by inhibiting protein biosynthesis in eukaryotic cells. Protein synthesis involves complex processes many of which are beyond the scope of this study. As an aid to understanding anisomycin's mode of action as an antibiotic, a brief description of the main concepts involved is presented, with an emphasis placed on the processes which anisomycin has a direct effect on.

1.2.1 Protein Synthesis in Eukaryotic Cells

Protein synthesis begins in the nucleus of a cell where transcription takes place. Transcription is the process by which RNA is formed from DNA, and occurs when an enzyme, RNA polymerase,[16] creates a transcription 'bubble' in which DNA is transiently unravelled.[17] RNA polymerase then controls the replication of one of the two unravelled strands of DNA by the complimentary base pairing of ribonucleotides with deoxyribonucleotides.[18] As the RNA polymerase travels down the DNA, the base pairs of the DNA recombine behind it and the DNA recoils (**Figure 2**).

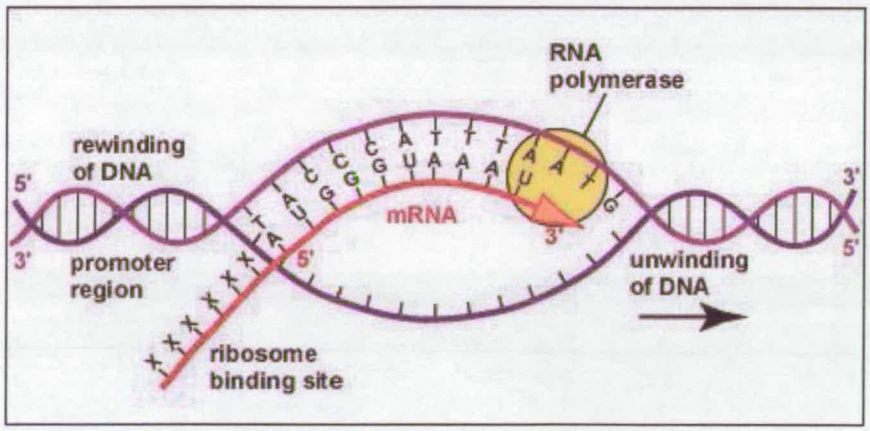

Figure 2 : Nuclear Transcription

The coding sequence on the RNA produced is interrupted at various points by noncoding tracts known as introns. These introns are 'edited out' by a process known as splicing[19] to produce a substance known as messenger RNA[20] (mRNA) that is responsible for the coding of a specific polypeptide at the ribosome of the cell.

mRNA is comprised of a series of codons. A codon is a sequence of three ribonucleotides that code for a specific amino acid[21] and base pair with anticodons in tRNA during the translation stage of protein synthesis. From **Table 1** it is noticeable that three of the codons (UAA, UAG, UGA) code for the termination of a peptide chain,[22] and one codon (AUG) codes for the initiation of a peptide chain. The table also shows that a single amino acid may have multiple codons, and when this occurs, the difference between codons is usually in the third base. The reason behind this lies in the anticodon. If the anticodon contains either a U, G, or I base, this base may weakly bond to two or more different nucleotides. The result of this is that multiple codons can code for a single amino acid.[23]

	U	C	A	G	
U	UUU = Phe UUC = Phe UUA = Leu UUG = Leu	UCU = Ser UCC = Ser UCA = Ser UCG = Ser	UAU = Tyr UAC = Tyr UAA = Stop UAG = Stop	UGU = Cys UGC = Cys UGA = Stop UGG = Trp	U C A G
C	CUU = Leu CUC = Leu CUA = Leu CUG = Leu	CCU = Pro CCC = Pro CCA = Pro CCG = Pro	CAU = His CAC = His CAA = Gln CAG = Gln	CGU = Arg CGC = Arg CGA = Arg CGG = Arg	U C A G
A	AUU = Ile AUC = Ile AUA = Ile AUG = Met	ACU = Thr ACC = Thr ACA = Thr ACG = Thr	AAU = Asn AAC = Asn AAA = Lys AAG = Lys	AGU = Ser AGC = Ser AGA = Arg AGG = Arg	U C A G
G	GUU = Val CUC = Val GUA = Val GUG = Val	GCU = Ala GCC = Ala GCA = Ala GCG = Ala	GAU = Asp GAC = Asp GAA = Glu GAG = Glu	GGU = Gly GCG = Gly GGA = Gly GGG = Gly	U C A G

Table 1 : The Universal Genetic Code[24]

When the mRNA reaches the ribosome the next phase of protein synthesis begins. Transfer RNA (tRNA),[25] (**Figure 3**), has a specific amino acid attached at its 3' end by an enzyme (aminoacyl-tRNA synthetase)[26] to produce aminoacyl-tRNA.

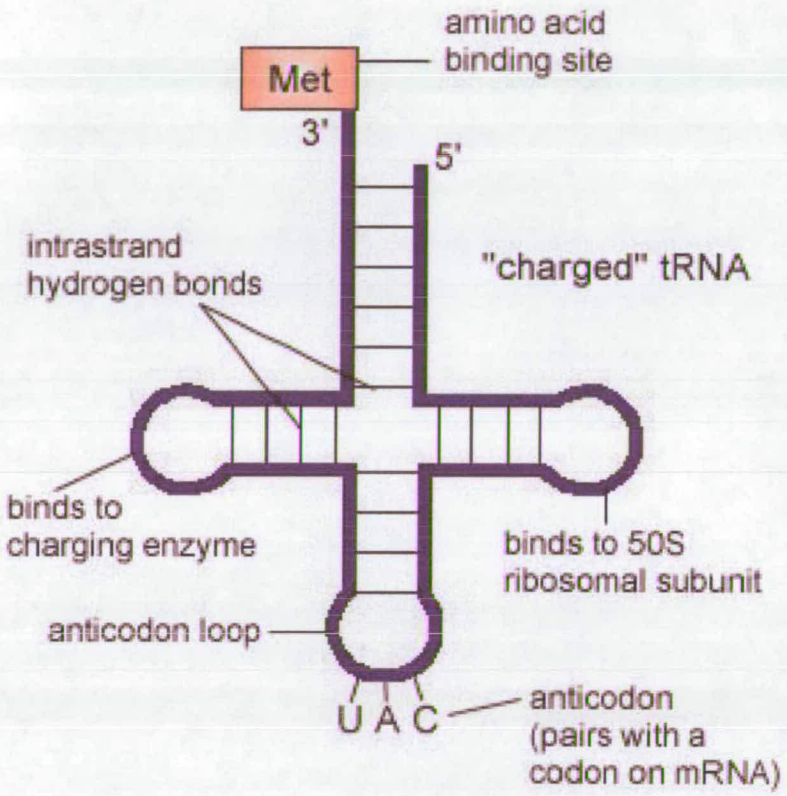

Figure 3 : The Structure of tRNA

The mRNA described earlier contains a short nucleotide sequence called a ribosome binding site and this binds to the 40S ribosomal subunit. The subunit begins reading the mRNA and when it encounters the AUG codon an initiation complex is formed comprising of the aminoacyl-tRNA and a group of proteins called initiation factors.[27] The aminoacyl-tRNA base pairs with the AUG codon and the initiation factors leave. A 60S ribosomal subunit[28] then attaches to the initiation complex to form an 80S ribosome.

The 80S ribosome consists of an acceptor site (A site) which is occupied by aminoacyl-tRNA, and a peptide site (P site)[29] occupied by tRNA to which is attached the peptide chain (peptidyl-tRNA). During peptide bond formation, the α-amino

group of the amino acid at the A site acts as a nucleophile, displacing the tRNA in the P site to form a peptide bond (**Figure 4**).[30,31] This reaction was initially thought to be catalysed by a protein called peptidyl transferase, however, recently it was found to be the ribosome that catalyses this reaction. The tRNA left at the P site leaves the ribosome through the exit site (E site) to be recycled,[32] and the peptidyl-tRNA, now at the A site, moves to the vacant P site[33] as the 80S ribosome translocates to the next codon on the mRNA. This process is repeated[34] until a stop codon on the mRNA is reached. At this point a group of proteins called release factors are produced which free the polypeptide from the peptidyl-tRNA, and the remaining 80S ribosome breaks down to be recycled.[35]

The discussion above describes protein synthesis in eukaryotic cells. Protein synthesis in bacterial cells is quite similar, with the only major difference being that the ribosome is comprised of 50S and 30S subunits, as opposed to the 60S and 40S subunits in eukaryotic cells.

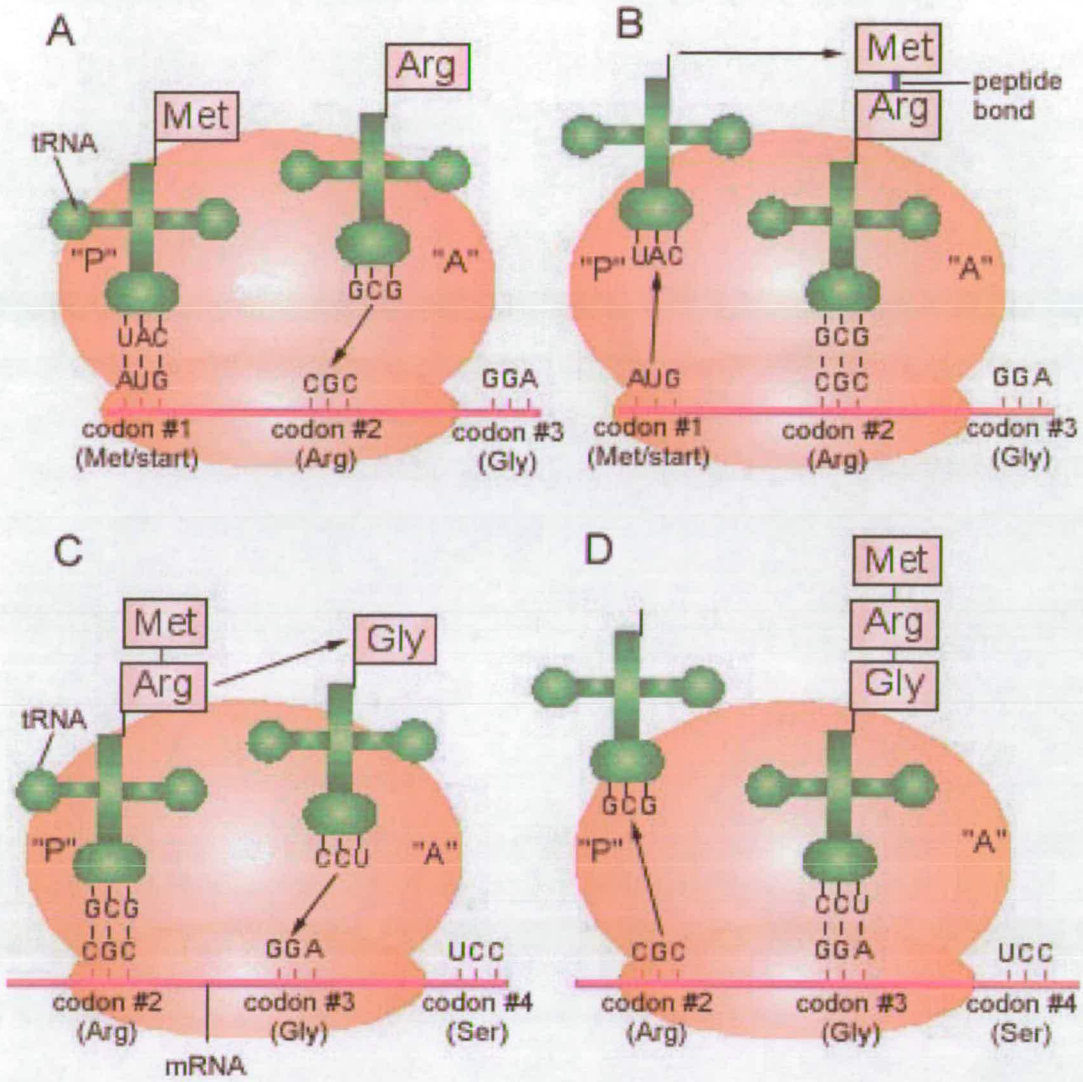

Figure 4 : Translation

1.2.2 Other Inhibitors of Protein Synthesis

Antibiotics may inhibit protein synthesis in a number of different ways. For example antimicrobials, such as the fluoroquinolones, inhibit normal nucleic acid replication. Similarly rifampin blocks transcription by inhibiting bacterial RNA polymerase. In the following section only the antibiotics that act on ribosomes, and therefore function in a similar manner to anisomycin, will be discussed.

Puromycin **1** is an antibiotic isolated from *Streptomyces alboniger*.[36] Its structure clearly resembles the 3' end of a charged aminoacyl-tRNA, but instead of containing an activated ester it contains an unreactive amide bond (**Figure 5**).[37] Puromycin acts by mimicking aminoacyl-tRNA **2**[38] and participates in all the usual chain elongation steps to produce peptidyl puromycin. However, peptidyl puromycin does not bind to the P site, or participate in translocation,[33] and instead dissociates from the ribosome prematurely, terminating protein synthesis.

Chloramphenicol **3** acts by binding to the 50S ribosomal subunit in bacteria[39] and preventing peptidyl transfer. This halts protein synthesis and stops the growth of the bacterial organism (**Figure 6**).

Tetracycline **4** and its group of antibiotics, inhibit protein synthesis in bacterial cells by attaching themselves to the 30S ribosomal subunit. This prevents aminoacyl-tRNA binding to the acceptor site, and stops protein synthesis.[40]

Linezolid **5** and other oxazolidinones function by binding to the 50S ribosomal subunit at a site close to that of chloramphenicol.[41] They act by stopping the 50S and 30S subunit coming together to form the 70S initiation complex,[42] and result in the inhibition of protein synthesis in bacteria.

The streptogramins **6** are comprised of two different components which work in a synergistic fashion. The first component (type A) binds to the 50S ribosomal subunit, and prevents the binding of aminoacyl-tRNA to the acceptor site in the ribosome. The conformational changes brought about by type A binding to the ribosome increase type B's binding affinity for the 50S ribosome,[43] and it too binds to the 50S ribosomal subunit. Type B then acts by blocking peptide bond formation, and promotes the premature release of incomplete polypeptides.[44]

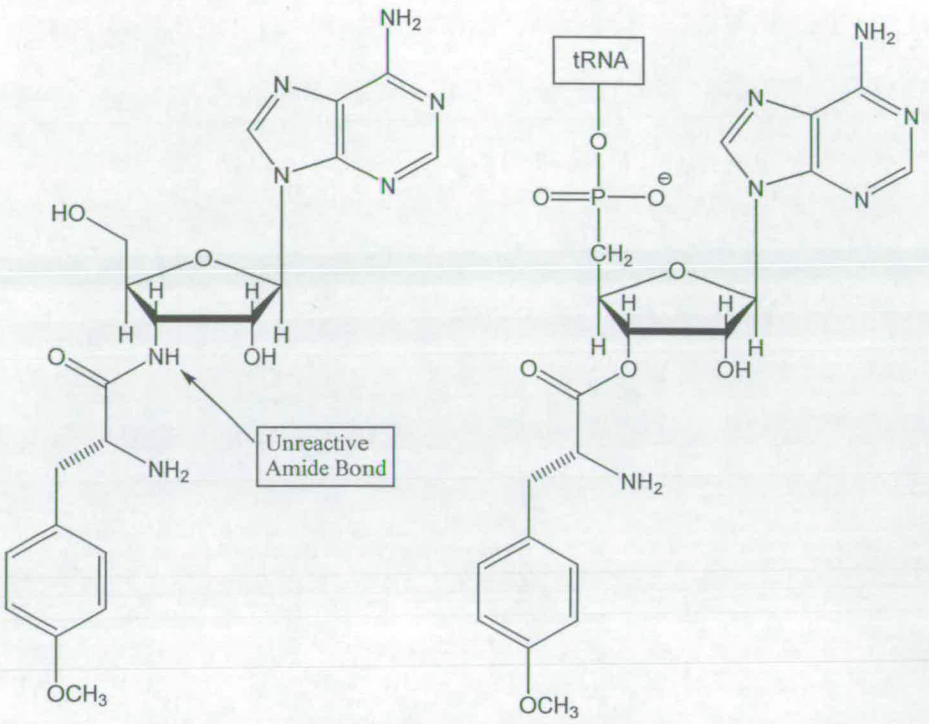

Figure 5 : A Comparison Between the 3' End of tRNA and Puromycin

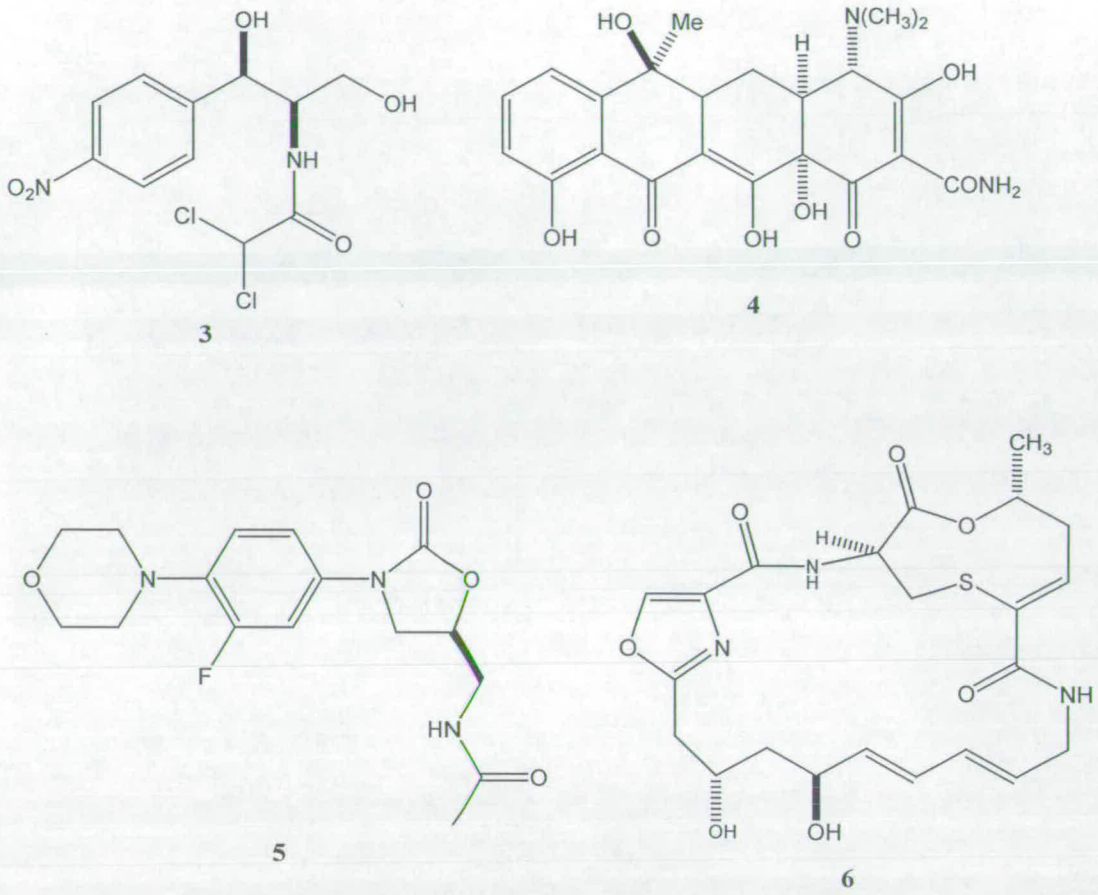

Figure 6 : Antibiotics Which Act on Ribosomes

1.2.3 Mode of Action of Anisomycin

During the 1960s research was carried out into discovering how anisomycin behaved as an antifungal antibiotic. It was found that when anisomycin was used at concentrations that caused 95% inhibition of protein synthesis, RNA synthesis was unaffected.[45] DNA synthesis however, was reduced but it was thought that this occurred as a direct consequence of the inhibition of protein synthesis.[46] It is clear therefore, that anisomycin does not inhibit protein synthesis by interfering with the transcription process.

Anisomycin has been found to inhibit the synthesis of haemoglobin from aminoacyl-tRNA in reticulocyte cell free extracts, and polyphenylalanine from phenylalanyl-tRNA in yeast extracts.[47] Therefore anisomycin must block some step after the formation of aminoacyl-tRNA. Since anisomycin has been shown to inhibit the puromycin-induced release of peptides from reticulocyte polysomes[45,48] and to block the formation of *N*-acetyl-(^{3}H)Leu-puromycin in both the fragment[Ψ] reaction[49,47(a)] and the puromycin reaction,[50] anisomycin may specifically block peptide bond formation on eukaryotic ribosomes.

Vazquez showed that anisomycin partially inhibits the interaction of the fragment CACCA-Leu-Ac with the donor site and the fragment CACCA-Leu with the acceptor site of the 60S ribosomal subunit.[47(a)] A year later he showed that anisomycin also inhibits the binding of phenylalanyl-tRNA and N-Ac-phenylalanyl-tRNA to the ribosomal A and P sites,[50] suggesting that anisomycin might inhibit peptide bond formation by affecting the correct positioning of the 3' termini of aminoacyl-tRNA and/or peptidyl-tRNA on the peptidyl transferase centre.[46]

[Ψ] In the fragment reaction both substrates involved in peptidyl transfer are replaced with simplified molecules. This is in contrast to the puromycin reaction where only the peptidyl acceptor is replaced.

1.3 Anisomycin As A Glycosidase Inhibitor

Glycosidases are a group of enzymes typically found in biological systems.[51] They function by catalysing the hydrolysis of glycoside bonds in carbohydrates. Not surprisingly, compounds such as iminosugars which inhibit glycosidases, display a number of biological properties. Castanospermine **7**[52] for example, has been shown to be effective against HIV, the agent responsible for AIDS.[53] Similarly swainsonine **8**[54] has been found to affect tumour growth by inhibiting the breakdown of oligosaccharides on cell surfaces, thereby effectively starving the tumour of sugar (**Figure 7**).[55]

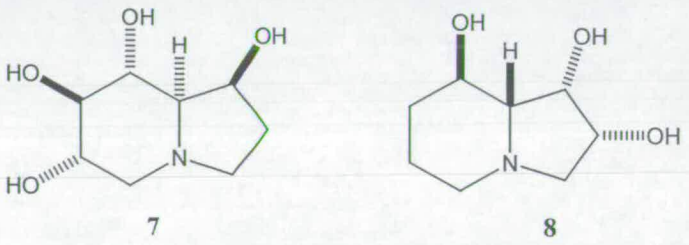

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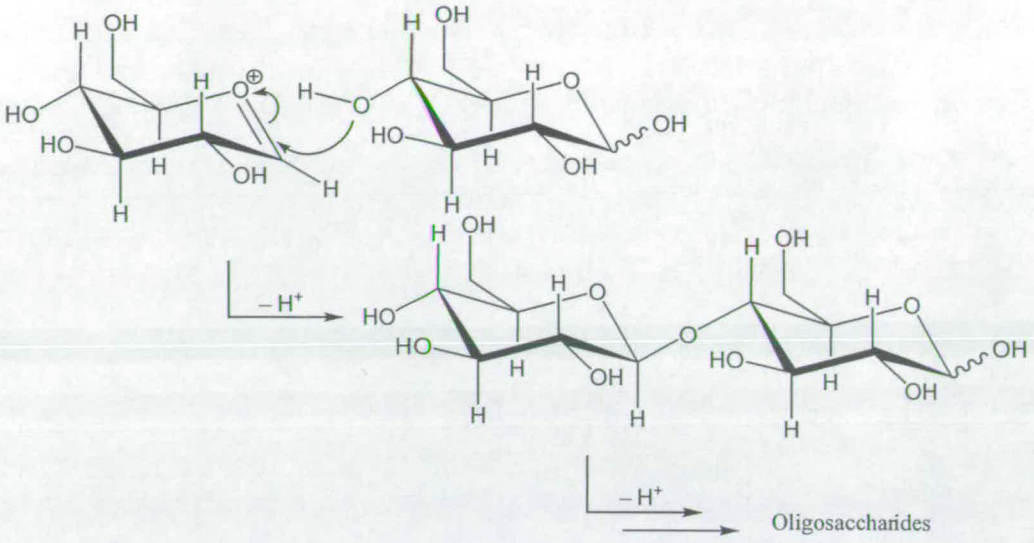
Figure 7 : Castanospermine and Swainsonine

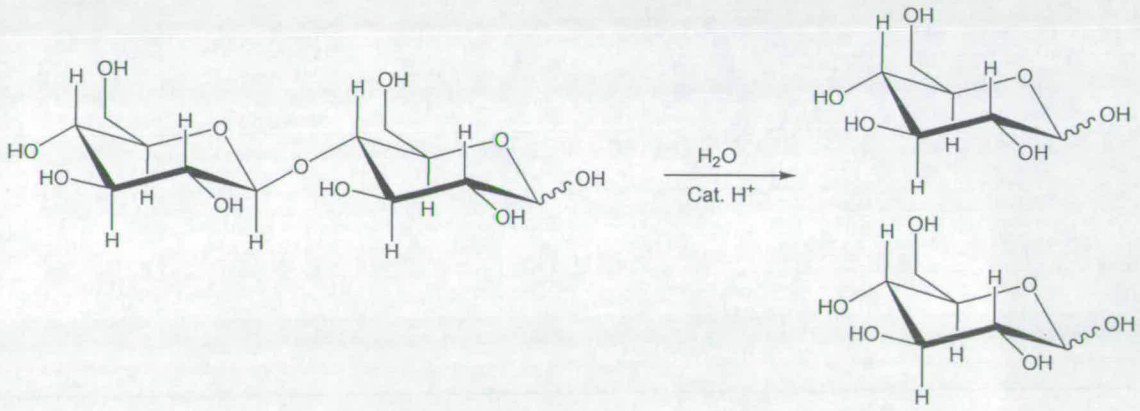
1.3.1 The Role of Glycosidase Enzymes

Disaccharides are produced when a hydroxyl group on one sugar unit reacts at the anomeric carbon centre on another, to form a glycoside bond that covalently joins the two monosaccharides (**Scheme 1**). Further reaction of this disaccharide with additional sugar units would allow the formation of oligo- and polysaccharides.



Scheme 1

Polysaccharides can be readily broken down to monosaccharides by treatment with dilute aqueous acids (**Scheme 2**). Under acidic conditions the glycoside bond is activated, allowing water to attack at the anomeric center forcing the glycoside bond to break.

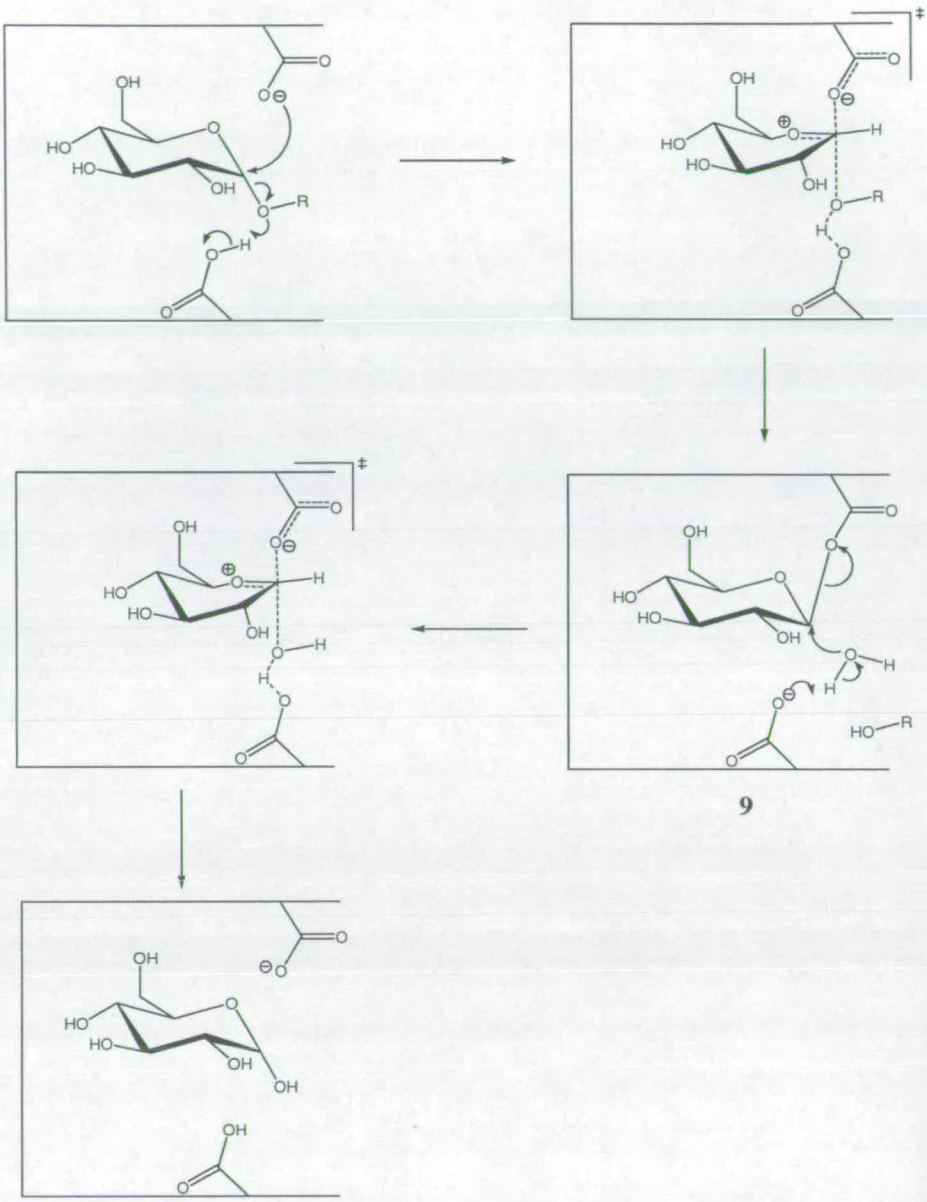


Scheme 2

In biological systems glycoside bonds are also cleaved by glycosidases. Glycosidases are a class of enzyme which act in a more controlled manner, by either hydrolysing glycoside bonds within the polysaccharide (endo), or at the ends of the sugar chain (exo)[56] using general acid/base catalysis.[57] Glycosidases show high

specificity towards the monosaccharides present in the substrate, and also towards the stereochemistry at the anomeric position. The hydrolysis occurs by one of two mechanisms giving rise to either inversion, or retention of the anomeric configuration.

In retaining glycosidases, the acid/base catalysis is brought about by the presence of amino acid residues on the wall of the enzyme's active site (**Scheme 3**). One of these acids acts as a general acid/base, whilst another behaves as a nucleophile/leaving group. The reaction is brought about by the activation of the glycoside bond, which then allows a second carboxylate group to attack the anomeric carbon centre. The reaction produces a covalent intermediate **9** and allows the release of the remaining polysaccharide chain (aglycone). In the next phase of the reaction, water present in the active site is activated towards the attack of the anomeric carbon. Its subsequent reaction displaces the carboxylate group, allowing the stereochemistry at the anomeric centre to be conserved in the resulting monosaccharide.[58,59]



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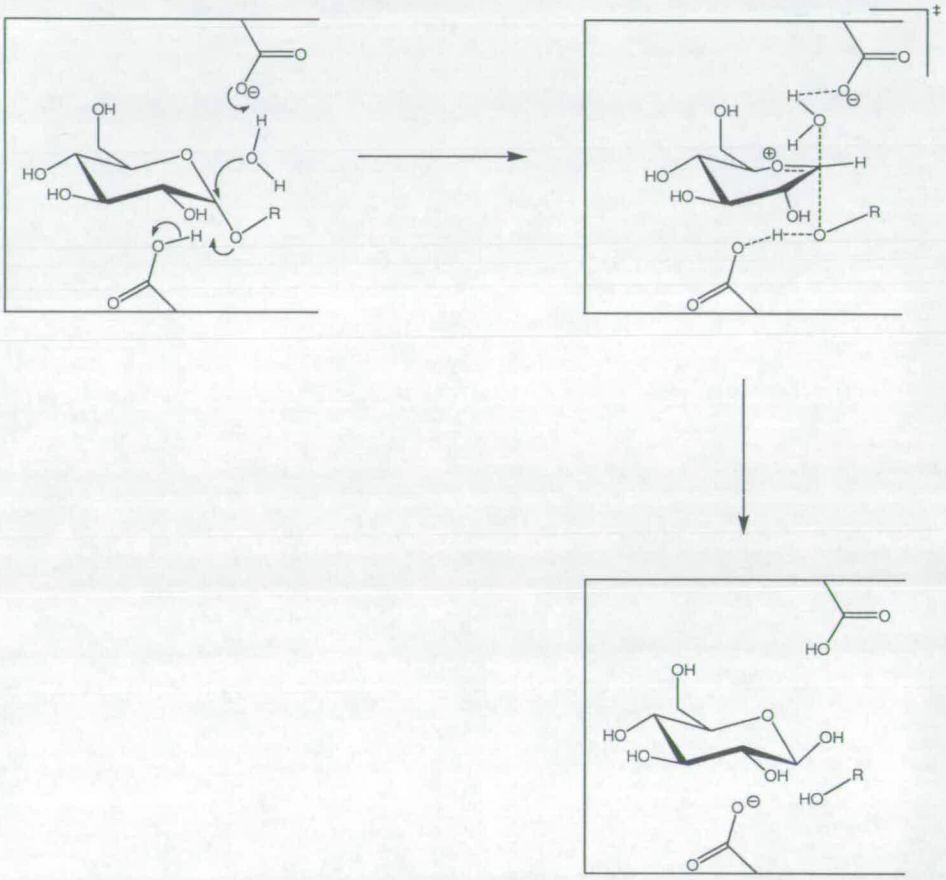
Scheme 3

With inverting glycosidases, the reaction again proceeds with the activation of the glycoside bond. However, rather than a carboxylate group in the active site acting as a nucleophile, it instead behaves as a base by activating trapped water. The attack by water leads to the direct displacement of the aglycone leaving the monosaccharide (glycone), with its stereochemistry at the anomeric position inverted (**Scheme 4**).[58]



Scheme 4

1.3.2 The Mode of Action of Glycosidase Inhibitors

For the inhibition of a glycosidase to occur, an inhibitor must bind to the active site of the enzyme in preference to a polysaccharide. It is clear therefore, that to produce an effective glycosidase inhibitor, it must be able to mimic the transition state produced in a glycosidase enzyme.

A closer study of the mechanism for both inverting and retaining glycosidases shows that a common transition state is formed, in which the anomeric carbon is sp^2 hybridized (**Figure 8**).[60] This change in hybridization gives rise to a half chair conformation. Therefore to produce an effective glycosidase inhibitor, it too must have this half chair conformation.

The inhibitory behavior of iminosugars has been attributed to the fact that they can fit into the active site of the enzyme by adopting an envelope or twisted half chair conformation.[59] Among other factors, the ability of a protonated iminosugar to form an ion pair with the carboxylate group of the catalytic site ensures that it binds in preference to other polysaccharides.[61]

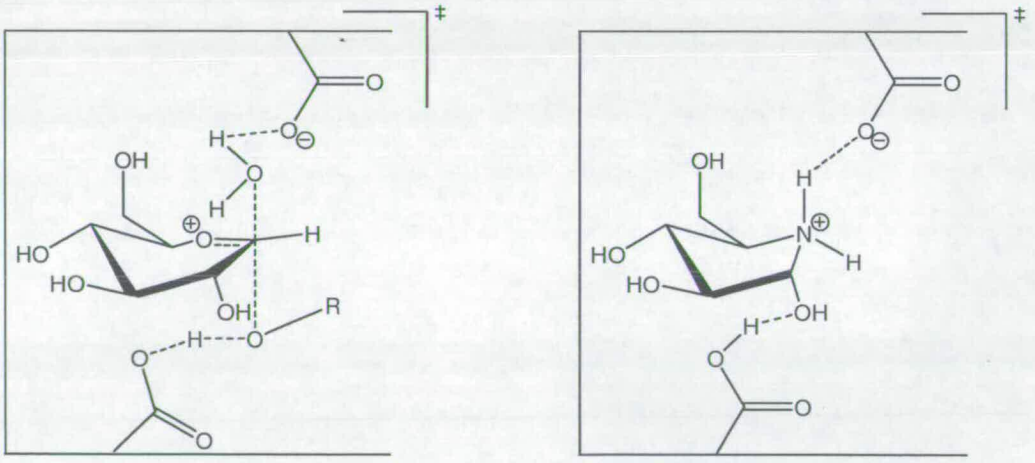


Figure 8 : A Protonated Iminosugar Mimicking a Polysaccharide in the Catalytic Site of a Glycosidase Enzyme

1.3.3 Pyrrolidine Iminosugars as Glycosidase Inhibitors

DAB-1 (**11**)[62] and DMDP (**12**)[63] are both examples of iminosugars which have successfully been used to inhibit glycosidases (**Figure 9**). DAB-1 has been shown to inhibit yeast α-glucosidase (IC_{50} 1.8×10^{-7} M)[64], where as DMDP has been shown to inhibit rat intestine lactase and bovine liver cytosolic *β*-glucosidase (IC_{50} 3×10^{-6} M).[65]

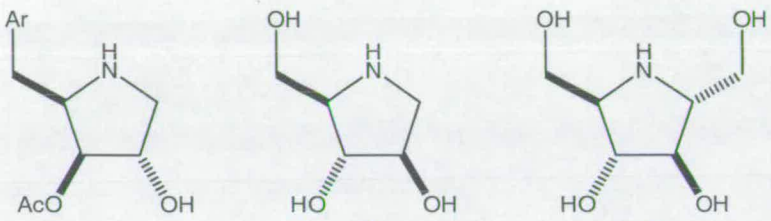


Figure 9 : A Comparison of Anisomycin with Two Iminosugars that Inhibit Glycosidase Enzymes

It has been shown that for the formation of glycoside bonds using *E. coli* β-galactosidase, the presence of a benzyl group at the anomeric position of xylopyranosides such as **13** increases the enzyme-acceptor binding, but also results in its partial inhibition (**Figure 10**).[66] It is anticipated therefore, that anisomycin with its *p*-methoxybenzyl group at the 2-position, and its potential to form an ion pair within the active site, will bind preferentially to glycosidase enzymes and function as an effective glycosidase inhibitor.

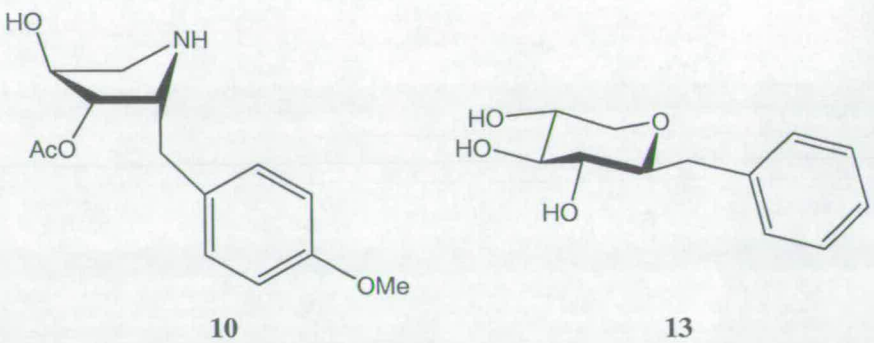


Figure 10 : A Comparison of Anisomycin with an Inhibitor of *E. coli* β-galactosidase

1.4 Anisomycin as a JNK/p38 Kinase Activator

MAP kinases (mitogen-activated protein kinase) are a group of protein serine/threonine kinases that are activated in response to a variety of extracellular stimuli. They function by mediating signal transduction from the cell surface to the nucleus.[67,68] The controlled regulation of MAP kinase cascades results in cell proliferation, differentiation, and cell repair/apoptosis.[69] However, unregulated activation can lead to oncogenesis.[70,71,72]

Anisomycin has been shown to strongly activate the JNK/SAPK (c-Jun NH_2-terminal kinase/stress activated protein kinases) and p38 MAP kinases in mammalian cells,[15] and results in the switching of cells from a resting state (G0) to an active state (G1). There are three major types of MAP kinase pathway in mammalian cells that have been reported. A brief discussion of these cascades is presented below.

1.4.1 Mitogen-Activated Protein Kinase (MAPK) Cascades

Cells membranes contain many different types of receptors to which growth factors and cytokines can bind. These receptors are comprised of two components. The first component is a ligand binding component, and is situated on the outside of the cell and ensures ligand specificity. The second component lies inside the cell and generates biological responses upon ligand binding. The biological response is determined by the type of receptor.

In the ERK1/ERK2 pathway the receptor is a protein tyrosine kinase (**Figure 11**). On ligand binding, tyrosine residues on the receptor are phosphorylated thereby providing a binding site for Grb. Grb is an adapter protein which binds to the receptor after it has bound to Sos (a guanine nucleotide releasing factor). These interactions bring Sos into close proximity with Ras (a membrane bound phospholipase), allowing Ras to become activated by the exchange of GTP for GDP.

The activated Ras protein phosphorylates the first signalling protein in the MAP kinase pathway called Raf. The active Ras protein then becomes deactivated through the hydrolysis of its GTP with the help of Ras-specific GAPs. The active Raf kinase then moves into the cytosol and phosphorylates a dual specificity kinase, MEK on serine (218) and serine (222).[73] The activated MEK, (the second signalling protein in the map kinase pathway) then phosphorylates MAP-K (ERK) on threonine (183) and tyrosine (185) and a fraction of this enzyme translocates to the nucleus to phosphorylate the transcription factor Elk-1. The proteins Raf, MEK and MAP-K are all deactivated after they have phosphorylated the next signalling protein in the MAP kinase pathway by specific phosphatases.

Once inside the nucleus Elk-1 begins to mediate changes in the expression of early response genes including *c-jun* and *c-fos*. Activated *c-jun* and *c-fos* then form a dimer which is an ideal DNA-binding protein that recognises specific DNA elements within promoter regions of genes. When the dimer binds to the promoter region of a gene, general transcription factors and RNA polymerases are produced allowing the transcription of downstream genes to occur. (*cf.* Section 1.2) The mRNA produced is transcribed into protein at the ribosome. This leads to an overall change in the amount of protein in the cell and gives rise to a change in the overall cellular behaviour.

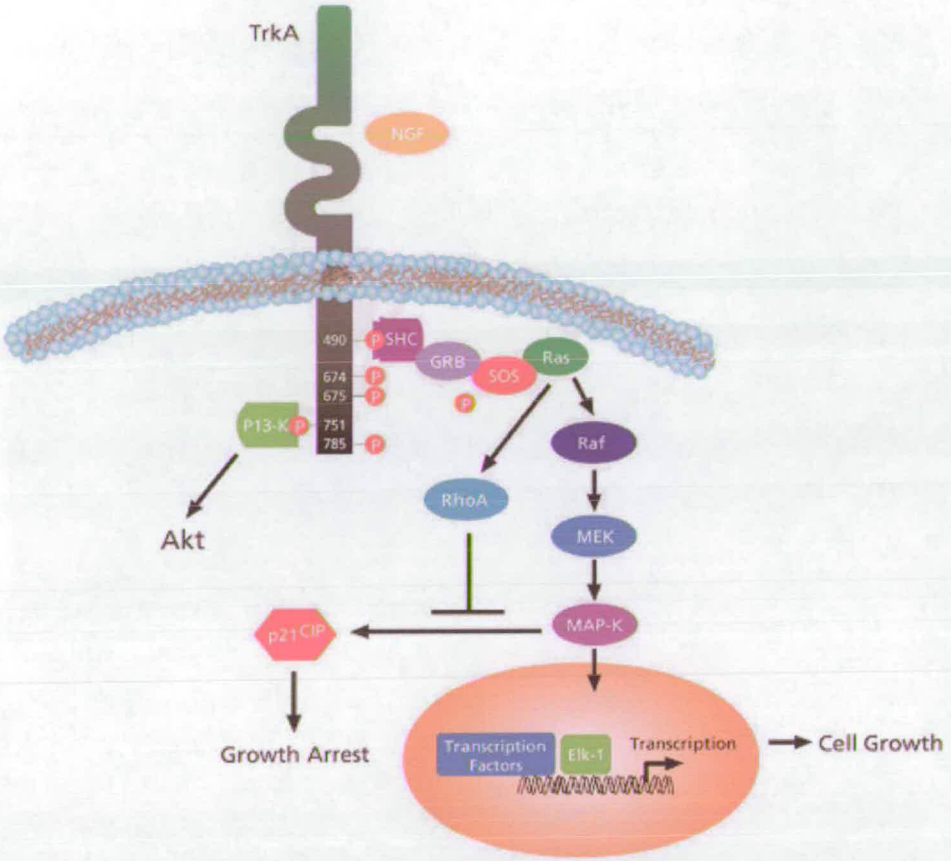


Figure 11 : The ERK1/ERK2 Pathway

The JNK/SAPK cascade is activated following exposure to UV radiation, heat shock, or inflammatory cytokines.[68,74] One mechanism of activation is *via* a receptor on the cell membrane. Once this receptor has been activated it transmits its message through G-proteins (GTP-binding proteins) to a membrane bound phospholipase, Rac.[67,74] Once this membrane bound phospholipase has been activated it binds to the PAK65 protein kinase phosphorylating it,[75] initiating a similar MAP kinases cascade to Elk-1. Thus PAK65 activates MEKK which in turn phosphorylates and activates MKK 4/7 at serine (219) and serine (223).[68] MKK 4/7 then activates JNK/SAPK1 allowing it to translocate to the nucleus. Once inside the nucleus it binds to the N-terminal region of c-Jun and phosphorylates it at serine (63) and serine (73).[76] The result is again the transcription of genes and eventually the formation of protein.

The p38 kinase is the newest member of the MAP kinase family.[68] Consequently much is still not known about the upstream steps in its activation. The kinase cascade is activated in response to inflammatory cytokines, endotoxins and osmotic stress.[72,77,78] The first known kinase to be activated is MMK3/6, a dual specificity kinase. This kinase activates the p38 kinase causing it to translocate to the nucleus and phosphorylate the transcription factor ATF-2.[79] The p38 kinase has also been found to activate MAPKAPK2, a kinase involved in the phosphorylation and activation of heat-shock proteins.[80]

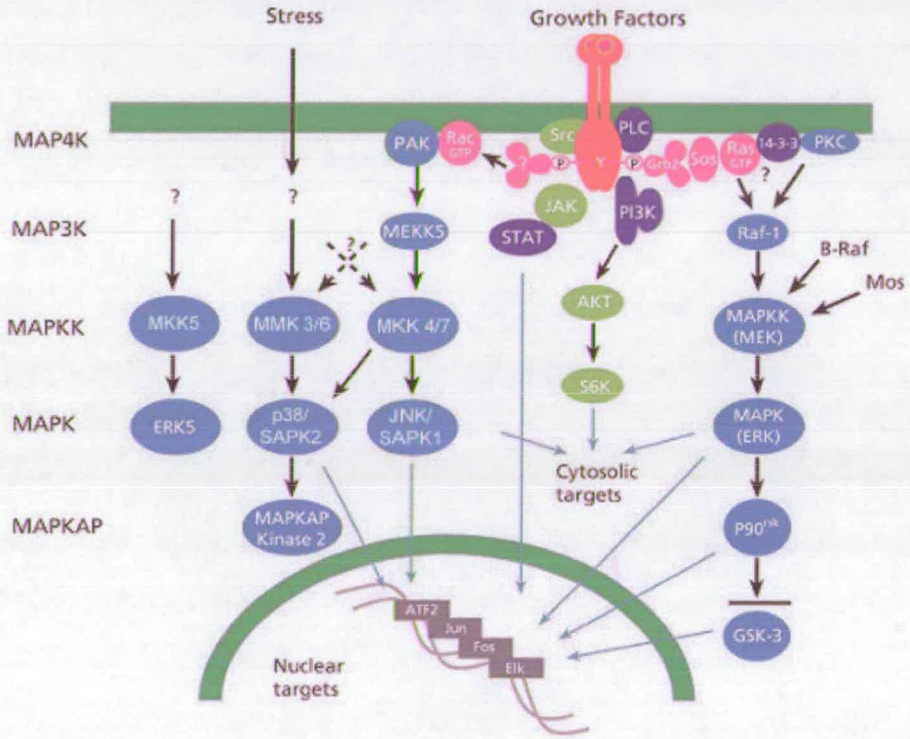


Figure 12 : Stress–Activated Kinase Pathways

Although much has been discovered about the MAP kinase pathways a lot still remains unclear. Even though it has been shown that the MAP kinase family can be separated into different pathways displaying a high degree of specificity and functional separation, there is still some degree of cross-talk between the different cascades (**Figure 12**). For example, MKK 4/7 not only activates JNK/SAPK but is also reported to activate p38.[81] Similarly, at the transcription level, ATF-2 is phosphorylated and activated by all three MAP kinases, whereas c-Jun and Elk-1 are phosphorylated only by ERKs and JNK/SAPK. However, despite all this, these pathways still result in transcriptional activity that is unique for a particular external stress.[82]

1.4.2 Anisomycin's Roles as a JNK/p38 Kinase Activator

Anisomycin has been found to activate both the JNK and p38 pathways at sub-inhibitory concentrations.[ξ] However the precise relationship between JNK/p38 activation and anisomycin is not yet known.

It has recently been discovered that peptidyltransferase inhibitors can trigger a ribotoxic stress response that activates the JNK1 MAP kinase.[83] It has therefore been proposed that anisomycin, itself a peptidyltransferase inhibitor, may activate the JNK pathway *via* a ribotoxic stress response rather than activating the kinase pathway directly.[84]

Anisomycin is known to bind to the 60S ribosome 300 times stronger than its deacetylated form.[85] Since deacetylanisomycin has little effect on protein synthesis or JNK1 kinase activation,[86] it is proposed that the ribotoxic stress response which activates the JNK1 kinase is produced when anisomycin binds to the 60S ribosomal subunit.

[ξ] At sub-inhibitory levels (typically 10 ng/ml) anisomycin activates the kinase pathways but does not inflict any toxic effects on cells through protein synthesis inhibition and may even cause a slight stimulation of protein synthesis, presumably due to its gene-inducing effect.

The arguments above suggest that a series of anisomycin analogues displaying a range of activity towards the JNK1 kinase, should display a similar pattern of activity towards peptidyltransferase inhibition. Surprisingly however, there are no such studies reported in the literature at the time of writing.

In a recent study of PC12 cells,[87] it was found that anisomycin directly targeted the p38-pathway in a Ras independent manner. It was also found that the JNK pathway was directly activated by anisomycin without the involvement of the p38 kinase. However, here it was thought that the Ras proteins did play some kind of role. Finally, activation of the ERK-pathway by anisomycin required not only the presence of a TrkA receptor and Ras protein, but also activation of the p38 kinase. This latter finding demonstrates a potentially important cross talk between the p38- and ERK-pathways (**Figure 13**).

All the effects of anisomycin described in the reactions above were inhibited by NAC, a scavenger of reactive oxygen species. The formation of oxygen free radicals by anisomycin and their mode of action still remains unclear, but their formation is clearly essential for activating the various MAPK pathways.

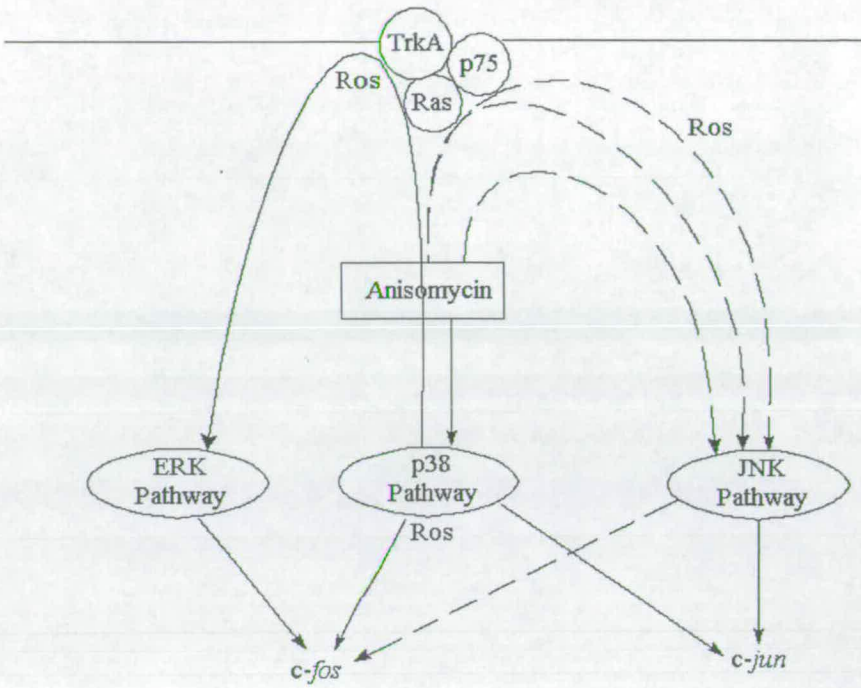


Figure 13 : Signal Transduction Pathways Affected by Anisomycin in PC12 Cells

It has been shown that anisomycin acts exactly like a signaling agonist in eliciting highly specific and virtually complete homologous desensitization of immediate-early (IE) gene induction and stress kinase activation.[88] Following the desensitization of a panel of IE genes (*c-fos*, *fos-B*, *c-jun*, and *jun-D*) using anisomycin, re-stimulation of the cells with a range of secondary stimuli gave selective responses.

It was found that re-stimulating the anisomycin-desensitized cells with anisomycin no longer activated the JNK/SAPK and p38 MAP kinase cascades. Attempts to activate the JNK/SAPKs with UV radiation and hyperosmolarity were also unsuccessful, and the activity of the p38 cascade was reduced to about 50% of its normal response. However, stimulating the cells with epidermal growth factors (EGF), tumor necrosis factor alpha (TNF-α), basic fibroblast growth factors (bFGF) and tetradecanoyl phorbol acetate (TPA) produced normal or augmented activation of these two kinase cascades.

These results suggest that anisomycin behaves like a true signaling agonist and that the anisomycin-desensitized signaling component is not involved in JNK/SAPK or p38 activation by EGF, bFGF, TPA but might play a significant role in UV- and hyperosmolarity-stimulated responses.

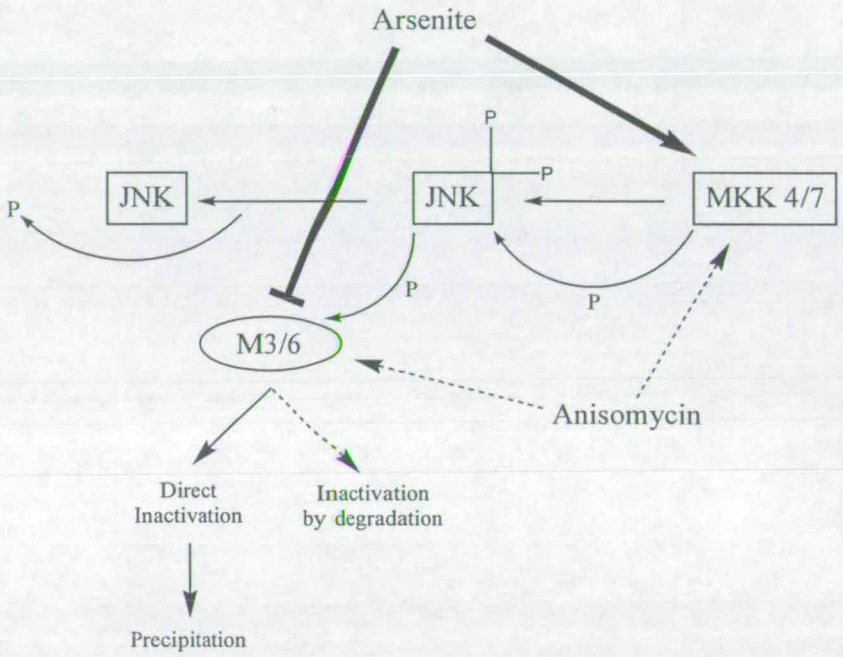


Figure 14 : Model of the Regulation of the M3/6 Phosphatase by Arsenite and Anisomycin

Ashworth has found that anisomycin activates the JNK pathway using two complementary processes.[89] It was found that anisomycin not only activated JNK/SAPK1 *via* its upstream kinase MKK 4/7, but also triggered the deactivation of the M3/6 (JNK-specific) phosphatase by ubiquitination and subsequent proteolytic degradation of the protein. This is in contrast to the effect of arsenite (and heat shock) which it is thought stimulates JNK phosphorylation by direct inactivation of M3/6 (**Figure 14**).

It is clear that this novel property of anisomycin to activate MAP kinases has an important role to play in discovering and understanding more about MAP kinase pathways. At present the role of anisomycin as a biological tool is hampered by the need to use sub-inhibitory concentrations. If an analogue of anisomycin could be developed which activated the JNK/p38 pathways without inhibiting protein synthesis at high concentrations, significant progress towards understanding the mechanisms involved in cell signaling could be made.

1.5 Summary of Chapter 1

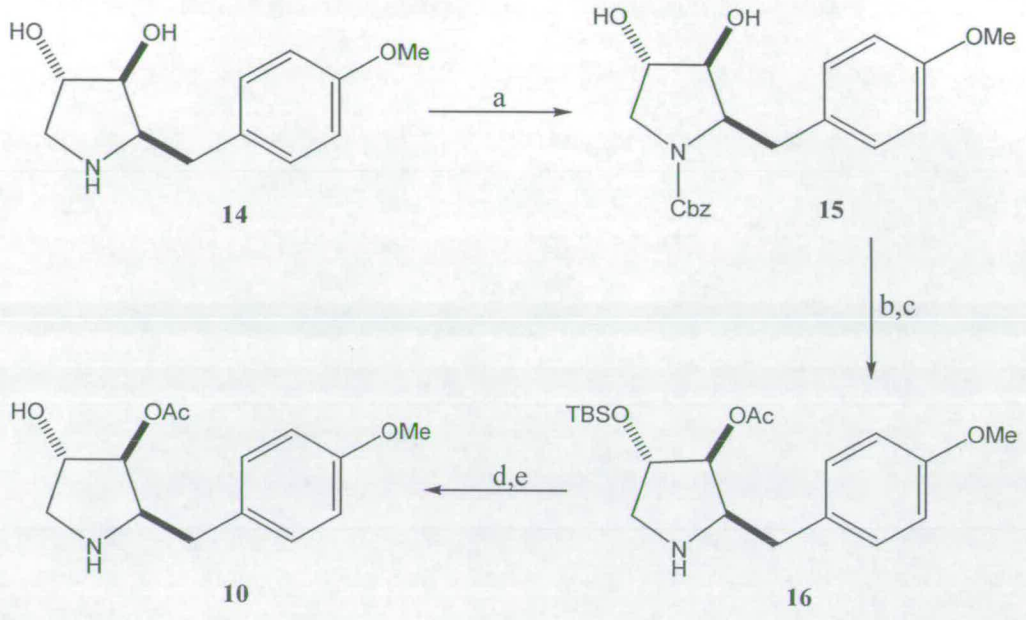
This chapter has highlighted the biological properties of anisomycin. Various biological systems that anisomycin shows activity towards have been discussed, as well as its proposed mode of action upon these systems.

Chapter 2 : The Synthesis of Anisomycin

2.1 Previous Syntheses of Anisomycin

Practical syntheses of anisomycin have met with increased interest during the last ten years due to reports of its high anti-tumour activity,[2] and its ability to activate the JNK and p38 MAP kinase pathways.[15] Over 20 syntheses of anisomycin or its biosynthetic precursor deacetylanisomycin **14** have been reported in the literature.[14,90] However, many of these syntheses suffer from a series of protection and deprotection steps in the latter stages, and the need for an efficient synthesis still remains.

Many early syntheses of anisomycin use deacetylanisomycin as a precursor, since its conversion to anisomycin by a series of 5 steps (**Scheme 5**) is well documented.[90(f)] The synthesis requires that a distinction be made between the C(3) and C(4) hydroxy functionality using various protecting groups. A yield of only 45% is obtained for this conversion, and reflects the difficulties involved in discriminating between the two secondary alcohols. Consequently, recent syntheses of anisomycin have tried to avoid this issue.



Reagents: (a) Na_2CO_3(aq.), $PhCH_2OCOCl$, CH_2Cl_2; (b) imidazole, TBSCl, DMF; (c) Ac_2O, pyridine; (d) TBAF, THF; (e) 10% Pd/C, H_2, EtOH.

Scheme 5

Anisomycin has been synthesised from various different starting materials. Although most syntheses have utilised the chiral pool, a couple of groups have approached the synthesis using achiral building blocks (**Figure 15**).

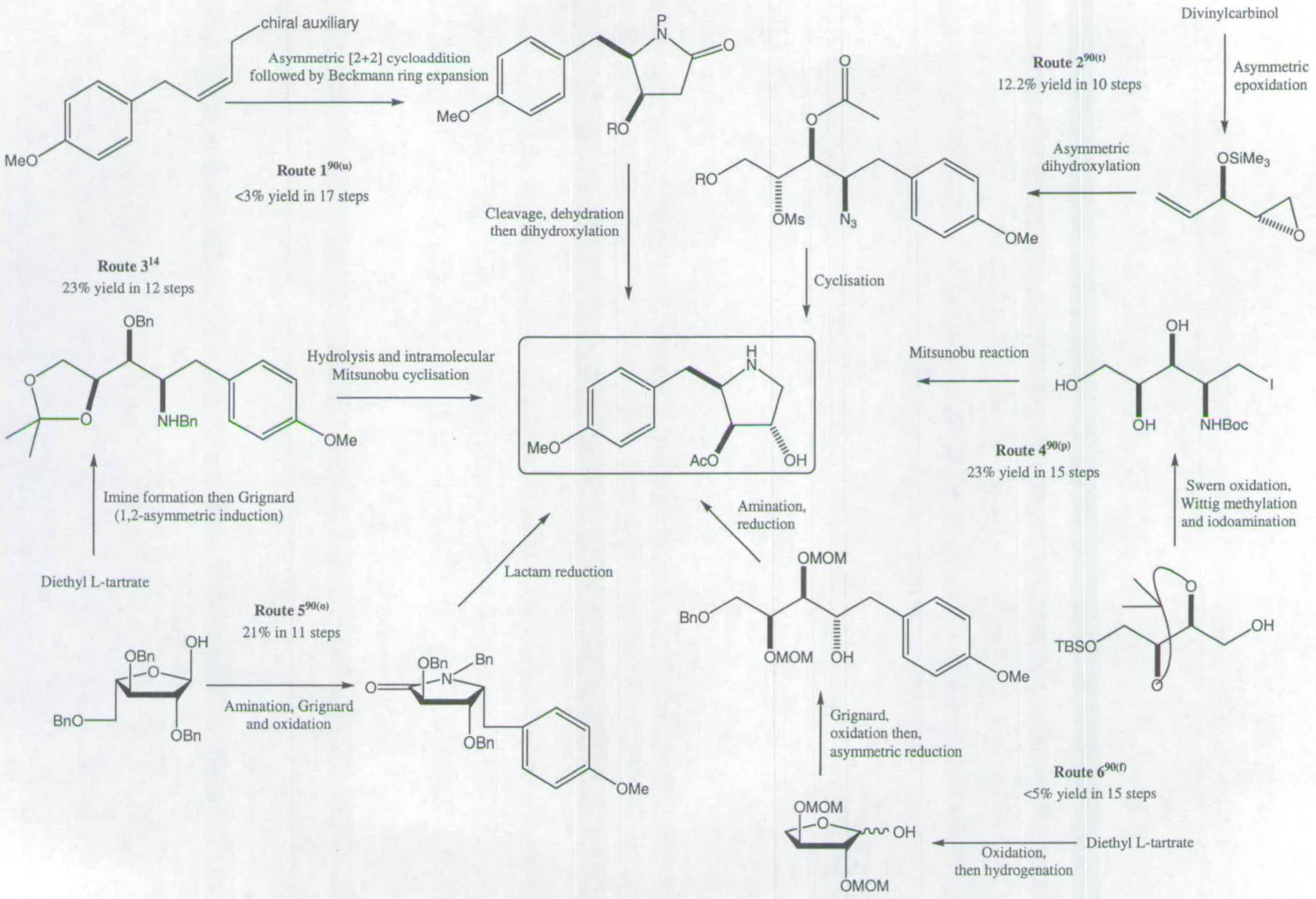
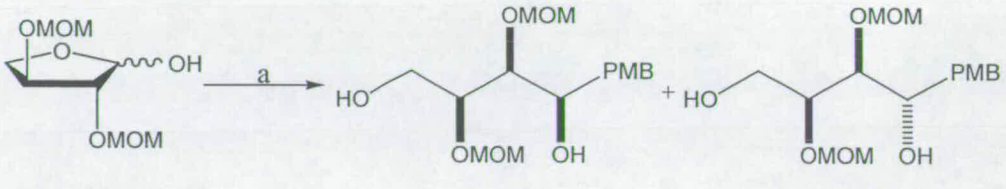


Figure 15 : Previous Syntheses of Anisomycin

The chiral functionality of anisomycin has been introduced in a variety of different ways (**Figure 15**). When the starting material is diethyl L-tartrate, the diol functionality is already in place. The *p*-methoxybenzyl group is introduced at the 2-position by creating an electrophilic centre and reacting it with 4-methoxyphenylmagnesium bromide (**Scheme 6**). This strategy is adopted in routes 3, 4 and 6. However in all three cases, it results in diastereomers being obtained which have to then be separated.



79% 21%

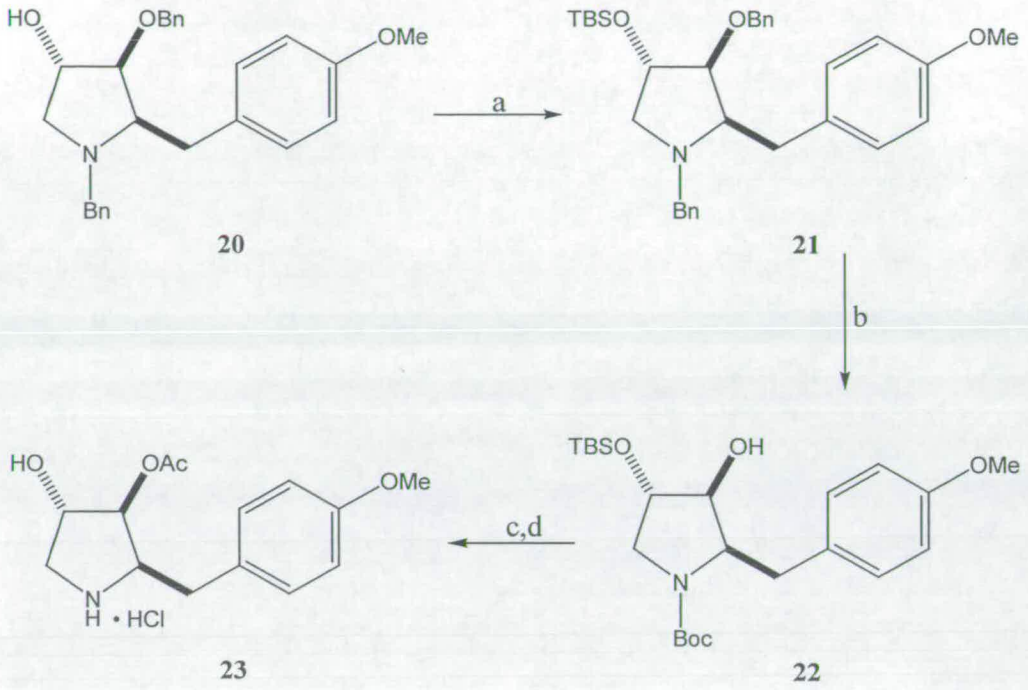
17 **18** **19**

Reagents: (a) PMBMgCl, THF.

Scheme 6

Another interesting point that is highlighted by these syntheses, is the struggle to differentiate between the C(3) and C(4) hydroxy functionalities. In diethyl L-tartrate there is no satisfactory way to discriminate between the two hydroxy groups. Consequently in routes 4 and 6, the same protecting group is applied to both alcohols. This results in the inevitable formation of deacetylanisomycin, whose conversion to anisomycin was described earlier.

Jäger did manage to discriminate between the two hydroxy groups by reducing one of the ester functionalities, and acetal protecting the resulting 1,2-diol (route 3). This protecting group strategy though is far from ideal, since it results in the wrong alcohol being protected in the latter stages of the synthesis (**Scheme 7**). This problem could have been overcome however, by protecting the 1,3-diol instead of the 1,2-diol.

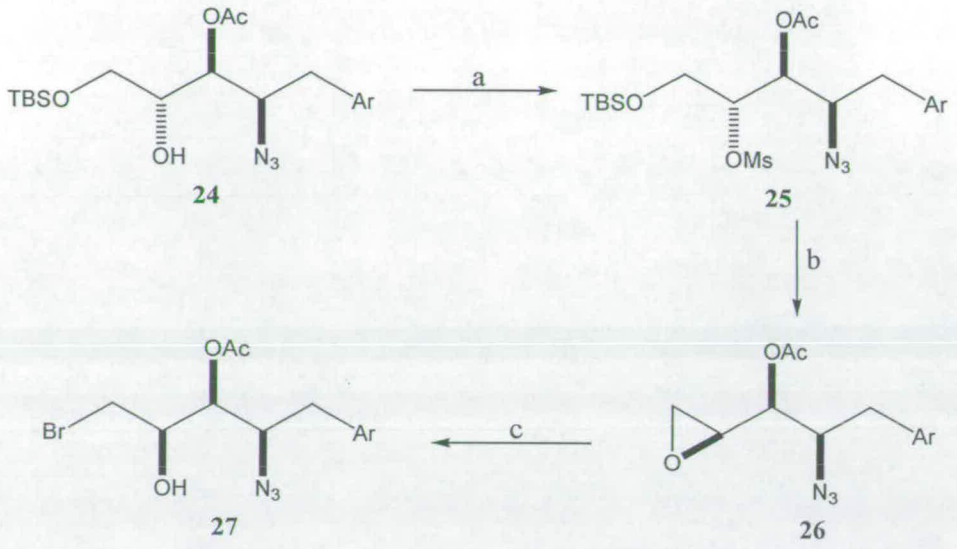


20 21 22 23

Reagents: (a) TBSCl, imidazole, DMF; (b) (i) $Pd(OH)_2/C$, H_2, Boc_2O, dioxane; (ii) HOAc; (c) Ac_2O, DMAP, pyridine; (d) HCl/dioxane (6 M), H_2O (1.1 eq).

Scheme 7

In a few syntheses of anisomycin, the wrong diastereomer has been obtained as a result of an asymmetric reaction. To rectify this, a series of three steps has often been employed which has not only increased the length of the syntheses, but also lowered the overall yield.



Reagents: (a) MsCl, pyridine, CH_2Cl_2; (b) 1.0 M n-$Bu_4N^+F^-$ THF solution, THF; (c) LiBr, HOAc, THF.

Scheme 8

In this synthesis by Lin (**Scheme 8**, route 2), an asymmetric dihydroxylation reaction produces the wrong diastereomer **24** as the major product. To correct this, the secondary alcohol is mesylated thereby making it susceptible to S_N2 attack by the primary alcohol. The epoxide formed now has the correct stereochemistry, and is opened by the reaction with LiBr to give the desired diastereomer **27**. A similar scenario exists in route 6, and is rectified in the same manner.

In an attempt to avoid the issues described above, Greene *et al* (route 1) used a chiral auxiliary to ensure that the correct stereochemistry was obtained. Overall, as a consequence, his synthesis required 17 steps and resulted in only a modest yield of 3%.

2.2 Retrosynthesis of Anisomycin

It was clear from the study of previous syntheses, that if we wanted to produce a short efficient synthesis of anisomycin we needed to distinguish between the C(3) and C(4) hydroxy groups at an early stage. In addition to this, if we wanted to improve upon all the syntheses to date, we needed to ensure that no protecting group manipulation was required in the later stages of the synthesis. Bearing all this in mind we arrived at the following retrosynthesis (**Figure 16**).

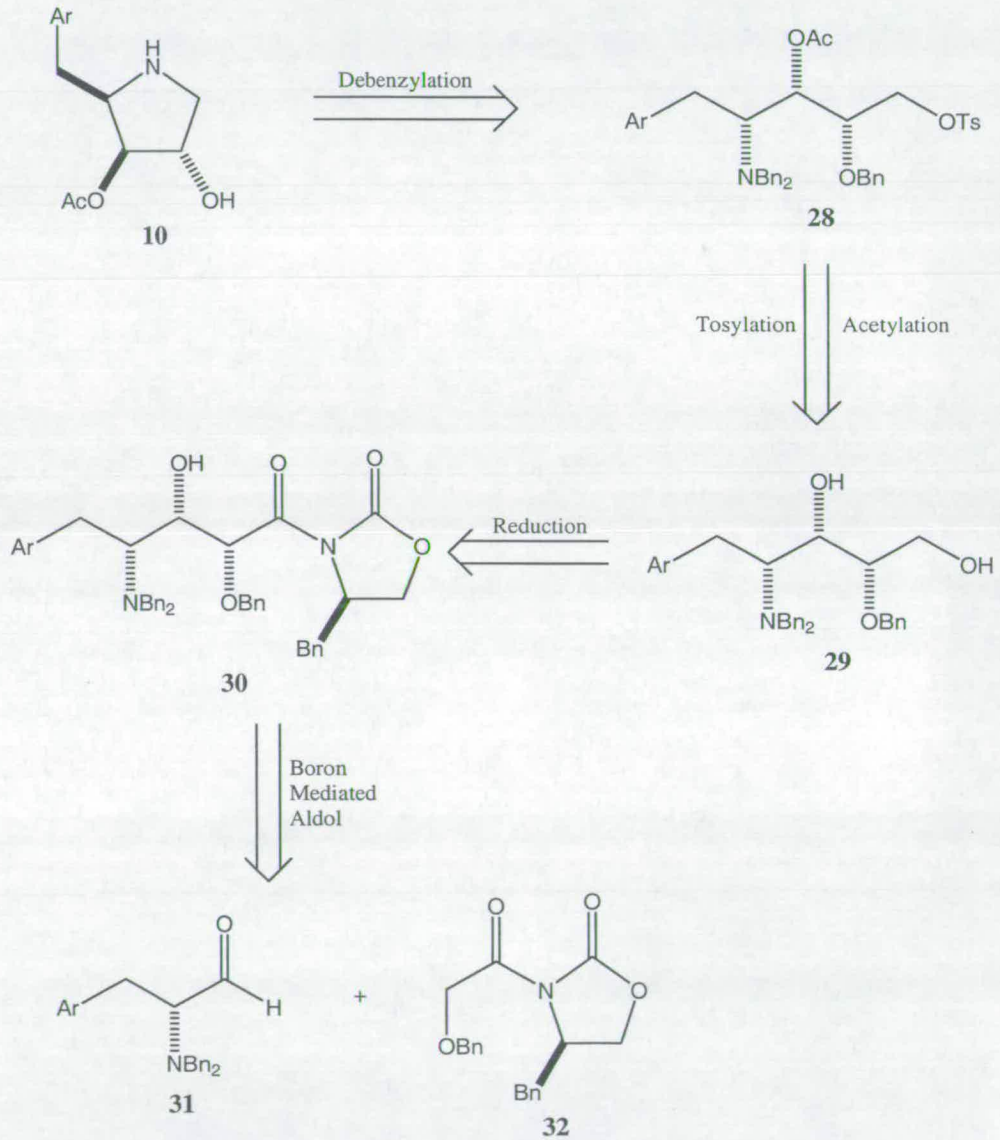
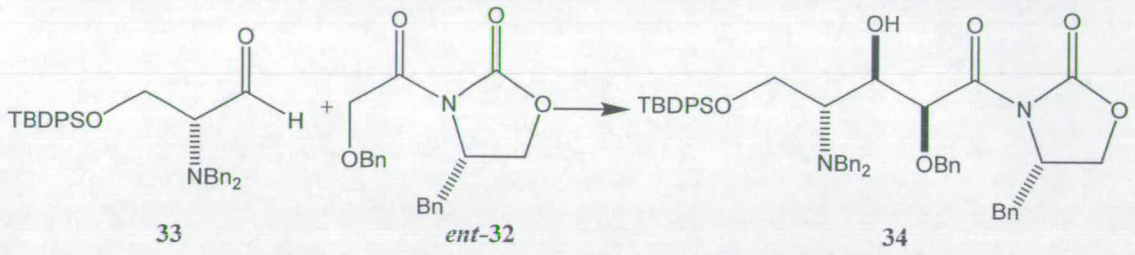


Figure 16 : The Retrosynthesis of Anisomycin

We were confident that deprotection of **28** would give anisomycin, since Lin had obtained anisomycin in a similar fashion.[90(t)] We anticipated that *para*-toluene sulfonyl chloride (TsCl) would selectively react at the primary alcohol in **29**, allowing the acetylation of the secondary alcohol to follow. We envisaged that aldol adduct **30** would readily react with lithium borohydride to give **29**, and we were optimistic that aldol adduct **30** could be obtained *via* the reaction of a tyrosine derived aldehyde with the acylated Evans' auxiliary.

Our optimism was based on the fact that DAB-1 and nectrisine have both been synthesised in the Hulme group by employing a highly diastereoselective *syn* glycolate aldol reaction with a serine-derived α-dibenzylamino aldehyde (**Scheme 9**).[91]



Scheme 9

We were hopeful that a similar approach using a tyrosine derived aldehyde **31** and the enantiomer of the acylated Evans' auxiliary *ent*-**32**, would allow us to access the aldol adduct **30** in good yield.

A comparison between the reduced aldol adduct **29**, and the intermediate **35** produced in Jäger's synthesis, shows that we too have successfully managed to discriminate between the two secondary alcohols (**Figure 17**). Our proposed synthesis also selectively protects the C(2)-OH of this intermediate rather than the C(3)-OH, thereby eliminating the need for protecting group manipulation in the latter stages, and thus fulfilling another of our objectives.

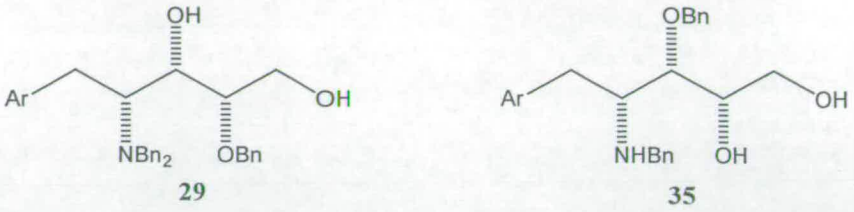


Figure 17 : A Comparison of Two Intermediates

2.3 Synthesis of a Tyrosine Derived *N,N*-Dibenzylamino Aldehyde

The *N,N*-dibenzyl protected serine derived aldehyde **33** has attracted considerable interest within the Hulme group over the last 5 years. This interest was sparked by Reetz,[92] who showed that additions to this aldehyde occur highly stereoselectively to give *anti* or Felkin-Anh[93] type products. The aldehyde has also been shown to be stable towards racemisation, therefore making its use in the aldol reaction extremely attractive.

We hoped to take this work a step further by developing a *N,N*-dibenzyl protected tyrosine derived aldehyde. We hoped this aldehyde would display the same selectivity and stability shown by its serine counterpart.

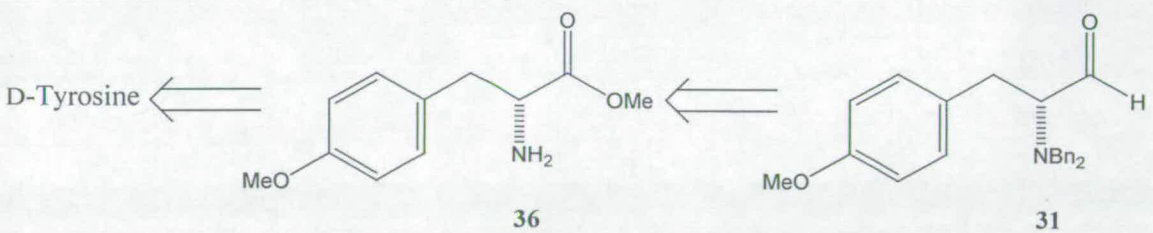
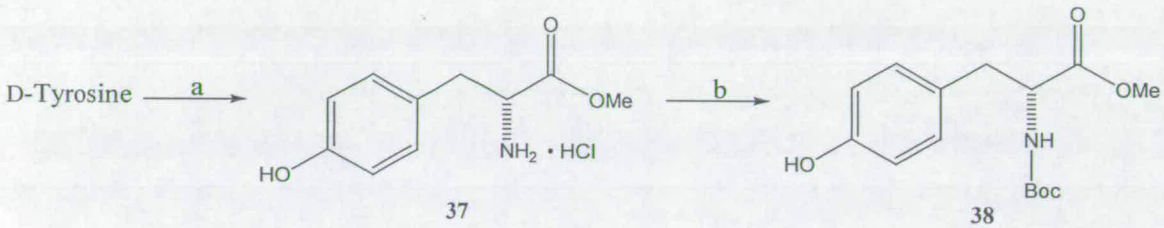


Figure 18 : The Retrosynthesis of Aldehyde 31

Our route to aldehyde **31** showed amine **36** to be a key intermediate (**Figure 18**). Although its enantiomer is commercially available, a synthetic route to **36** is still required.

Initial efforts focused on the synthesis of **36** from the *N*-Boc protected tyrosine methyl ester **38** using a method employed by Snider.[94] Therefore, **38** was prepared by converting D-tyrosine to its methyl ester hydrochloride salt **37** quantitatively using acetyl chloride and methanol (**Scheme 10**). The amine was then selectively protected using di-*tert*-butoxy carbamate in the presence of sodium bicarbonate to give **38** in excellent yield.



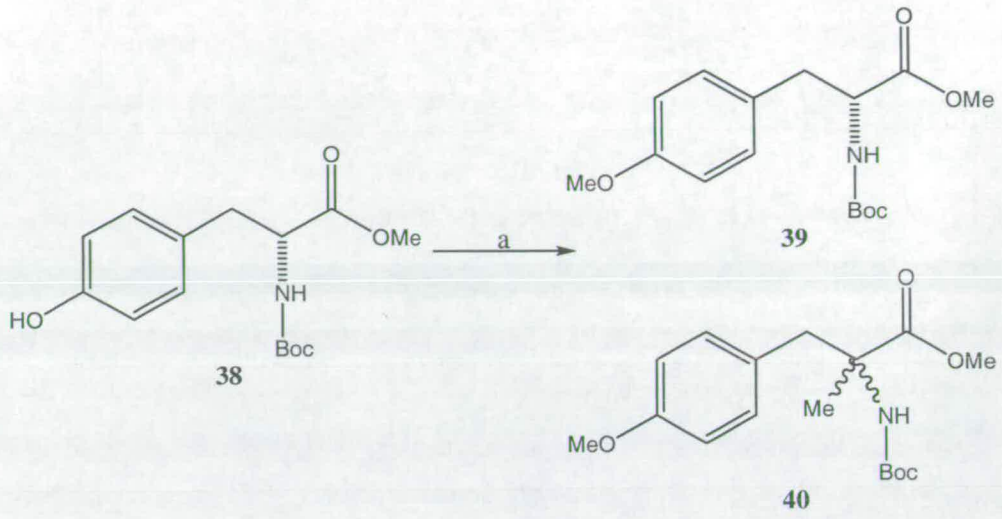
Reagents: (a) MeOH, AcCl (100%); (b) Boc_2O, $NaHCO_3$, EtOH (99%).

Scheme 10

Treatment of **38** with potassium hydroxide and methyl iodide as described by Snider, methylated the phenol as expected. However the presence of:

- A quaternary carbon instead of a methine carbon at 70.2 ppm in the ^{13}C NMR.
- An AB instead of an ABX system in the ^{1}H NMR.
- A parent ion at 402 mass units in the FAB mass spectrum.

suggested that methylation α to the carbonyl group had also occurred giving rise to a mixture of both **39** and **40** (**Scheme 11**).

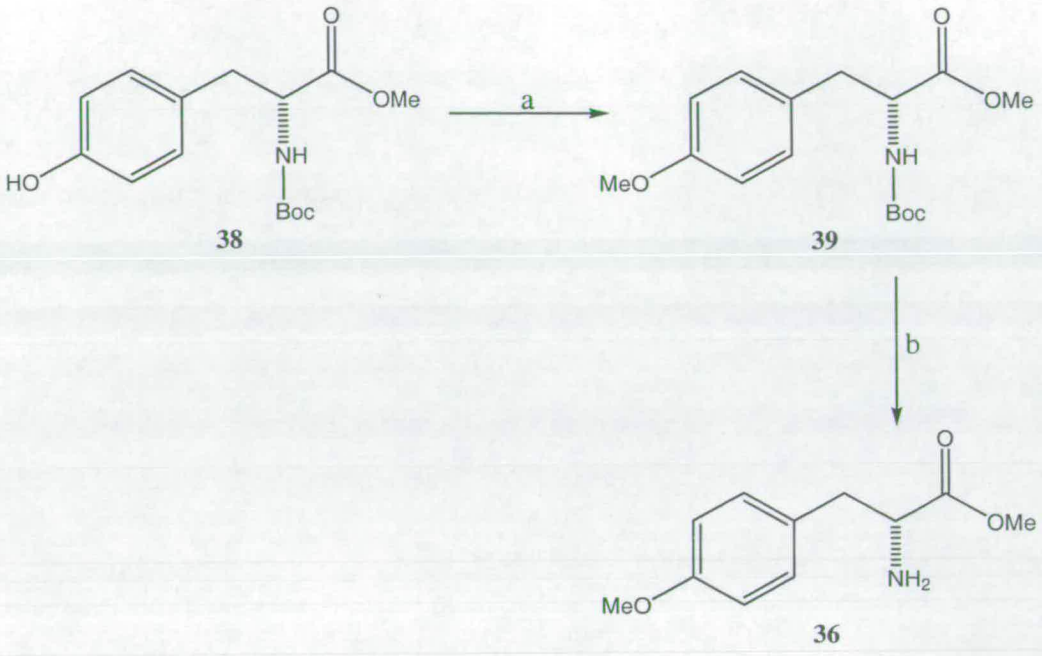


Reagents: (a) MeI, KOH, DMF.

Scheme 11

It was concluded that potassium hydroxide was not only removing the pheolic proton, but also the proton α to the carbonyl group, thereby allowing both centres to be alkylated. It was thought that a weaker base would eliminate this problem and allow the selective methylation of the phenol.

Consequently, phenol **38** was treated with methyl iodide in the presence of potassium carbonate to give **39** in excellent yield. With no trace of the di-methylated material being observed, the Boc group was removed using trifluoroacetic acid to give the free amine **36** (**Scheme 12**).

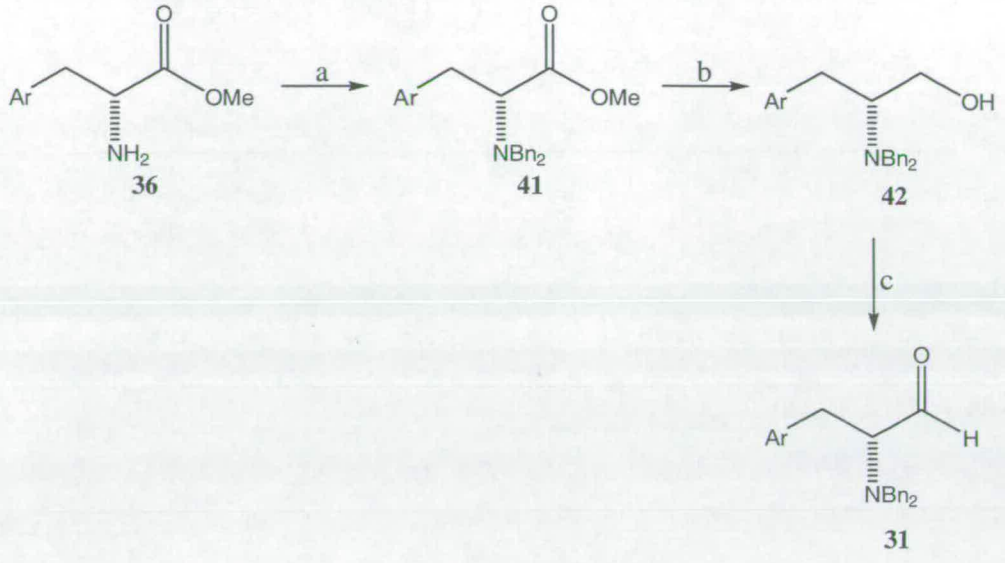


38 39 36

Reagents: (a) MeI, K_2CO_3, DMF (97%); (b) TFA, CH_2Cl_2 (100%).

Scheme 12

The free amine produced was *N,N*-dibenzylated using benzyl bromide in the presence of potassium carbonate, and reduction of **41** with $LiBH_4$ gave amino alcohol **42**. Finally oxidation of the alcohol using Swern[95] conditions gave aldehyde **31** quantitatively, and as a single spot by t.l.c. (**Scheme 13**). This proved important, as similar aldehyde's have been shown to be unstable to silica gel,[96] and it allowed us to use the aldehyde crude in subsequent reactions without the need for any chromatography.



Reagents: (a) BnBr, K_2CO_3, CH_3CN (95%); (b) $LiBH_4$, MeOH, Et_2O (87%); (c) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2 (100%).

Scheme 13

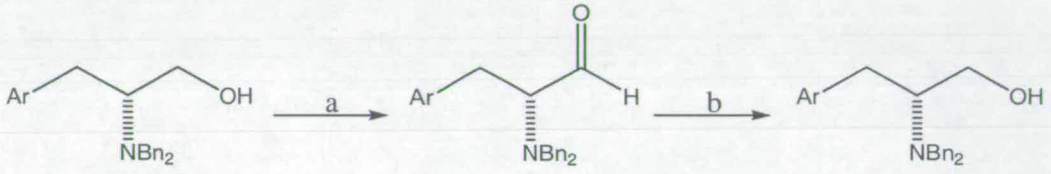
2.3.1 Enantiomeric Excess and Optical Stability of Amino Aldehyde

We were concerned about the enantiopurity of our amino alcohol. This concern was brought about by the ease with which racemisation occurred when alkylating phenol **38** in the presence of potassium hydroxide. To allay our fears and determine that racemisation had not occurred again, we decided to test the optical purity of the amino alcohol.

A racemic synthesis of amino alcohol **42** was carried out. The racemate obtained was analysed using reverse phase chiral HPLC (Chiracel OD-H column; solvent 10% propan-2-ol in hexane) in order to optimise column conditions and ensure good baseline peak separation. The single enantiomer was then analysed in the same manner, and reassuringly, showed that no appreciable racemisation had occurred. (material >98% ee, **Appendix A**).

In an effort to determine whether aldehyde **31** was prone to racemisation, its optical stability was tested indirectly *via* the alcohol, since it has been shown in the literature that aldehydes similar to **31** are unstable to silica gel.[96]

Samples of aldehyde **31** were synthesised in the usual manner from alcohol **42** using Swern conditions. The aldehyde **31** was then treated immediately with DIBAL-H at –78 °C, to regenerate **42** (**Scheme 14**). The alcohol obtained was analysed by chiral HPLC using the same conditions described earlier. The results again showed that no significant racemisation had occurred (material >98% ee). Thus, aldehyde **31** had been produced with high optical purity in seven steps and 80% overall yield from D-tyrosine.



42 **31** **42**

Reagents: (a) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2 (100%); (b) DIBAL-H, toluene (90%).

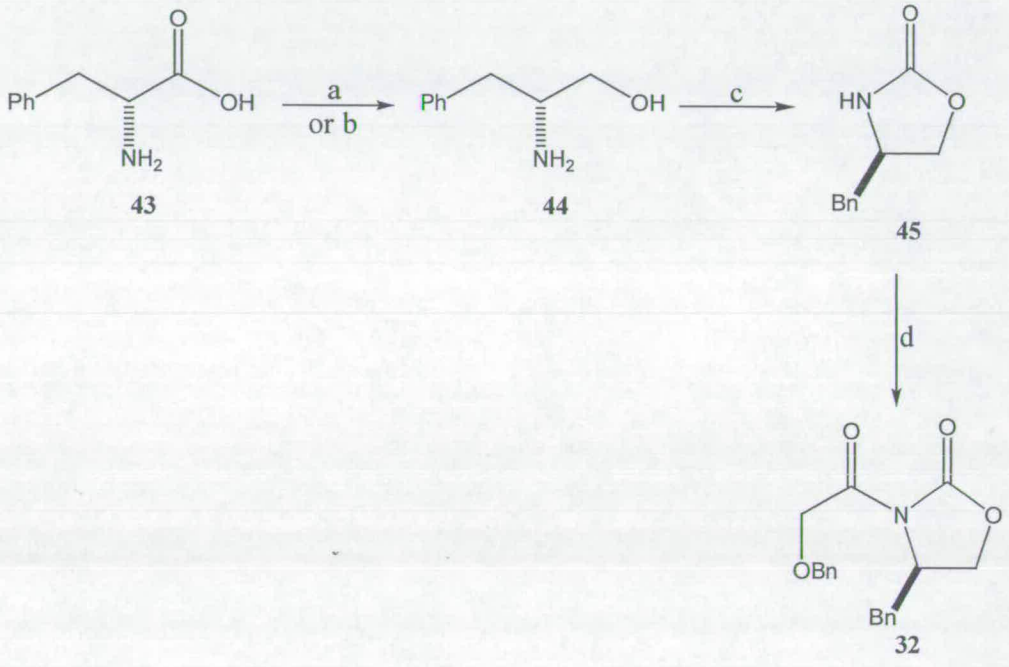
Scheme 14

2.4 Boron Mediated Aldol Reaction

2.4.1 Synthesis of the Glycolate Chiral Auxiliary

With amino aldehyde **31** successfully synthesised our attentions turned to the aldol reaction. Studies within the Hulme group and by others[97] have shown the glycolate equivalent **32** to be highly effective in aldol reactions. An advantage of the auxiliary to us, in our synthesis, was the *O*-benzyl protecting group. If successfully incorporated into our substrate, the *O*-benzyl protecting group would offer us the option of a global deprotection step in the latter stages, thereby increasing our synthesis' efficiency.

The glycolate equivalent **32** was prepared in 3 steps from D-phenylalanine *via* the Evans' auxiliary **45**. Thus D-phenylalanine was either treated with $NaBH_4$ using conditions described by Meyers,[98] or with TMSCl and $LiBH_4$ using a method described by Quagliato.[99] The amino alcohol obtained was then condensed with diethyl carbonate to give the Evans' auxiliary in good yield. Finally, treatment of the auxiliary with *n*-butyllithium followed by benzyloxyacetyl chloride gave glycolate equivalent **32** in excellent yield (**Scheme 15**).



Reagents: (a) $NaBH_4$, I_2, THF, KOH (60%); (b) $LiBH_4$, TMSCl, THF (95%); (c) K_2CO_3, $(EtO)_2CO$ (83%); (d) $BnOCH_2COCl$, BuLi, THF (89%).

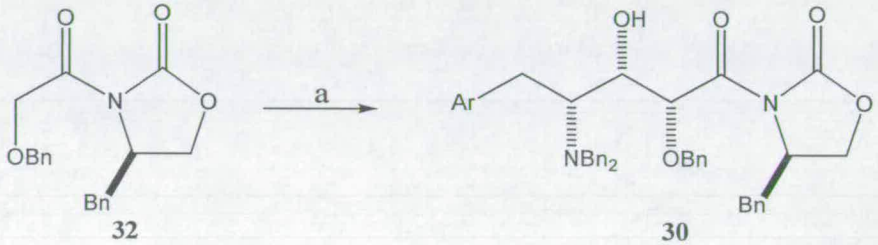
Scheme 15

2.4.2 Formation of the 'Mismatched' Aldol Adduct

With both coupling partners in hand, we were now ready to attempt the aldol reaction. Formation of the Z-boron enolate was carried out using standard conditions (Bu_2BOTf, NEt_3, -78 °C → 0 °C) and reacted with freshly prepared aldehyde **31** to give aldol adduct **30** as the major diastereomer. A diagnostic peak in the ^1H NMR at δ 4.89 (d, *J* 1.6 Hz) representing the $C_{2'}$ proton, suggested that the *syn* aldol product

had been obtained.[Ψ] Consequently aldol adduct **30** was tentatively assigned the 'mismatched' structure shown below (**Scheme 16**).

Trace amounts (<5 mg) of two other inseparable diastereomers were also obtained. Again, the diagnostic peak for a *syn* aldol adduct was clearly visible in the ^{1}H NMR at δ 5.06 (d, *J* 3.3 Hz), suggesting that the other *syn* aldol adduct had been isolated, along with one of the two possible *anti* diastereomers.



32 **30**

Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) **31**, (75%).

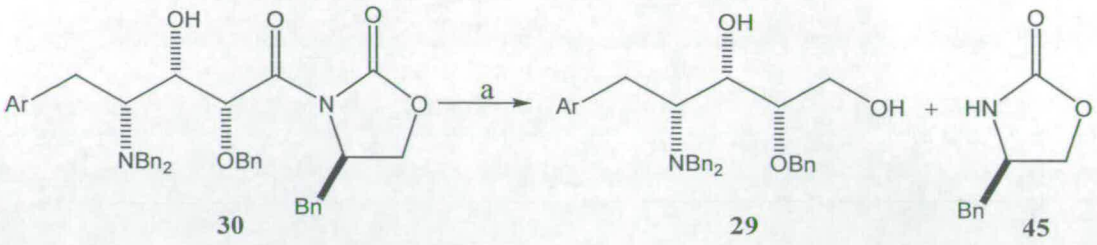
Scheme 16

A full investigation into this and other asymmetric glycolate aldol reactions is presented in chapter 4.

2.5 Synthesis of Deacetylanisomycin

In an attempt to determine the relative stereochemistry of aldol adduct **30**, we set about the synthesis of deacetylanisomycin. Thus aldol adduct **30** was reduced with $LiBH_4$ in the presence of methanol to give diol **29** in 80% yield. The Evans' auxiliary was also recovered, with a moderate yield of 60% (**Scheme 17**).

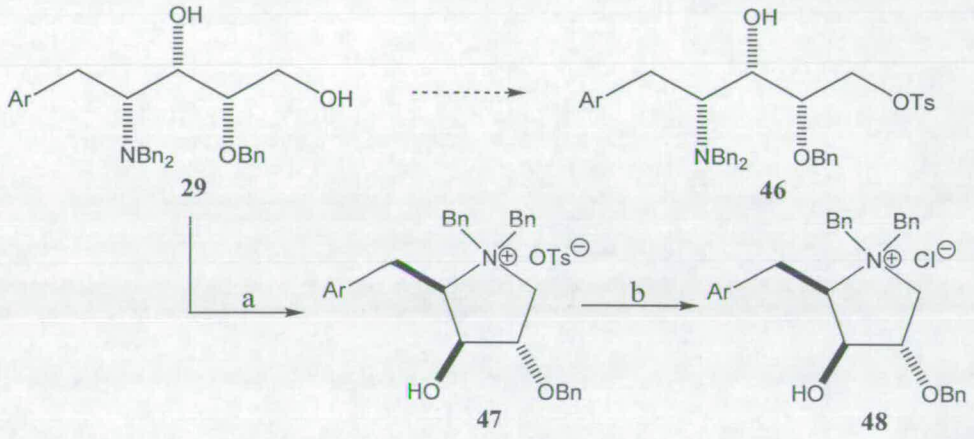
[Ψ] In solution a β-hydroxy ketone exists in a chair-like conformation as a result of intramolecular hydrogen bonding. The bulk long chain adopts a more favoured equatorial position and the dihedral angle between α and β protons is ~60° for the *syn* arrangement and ~180° for the *anti* arrangement. As a result, the Karplus relationship predicts vicinal coupling constants to be ~3-5 Hz for *syn* and ~7-12 Hz for *anti* aldol adducts.



Reagents: (a) $LiBH_4$, CH_3OH, THF (80%).

Scheme 17

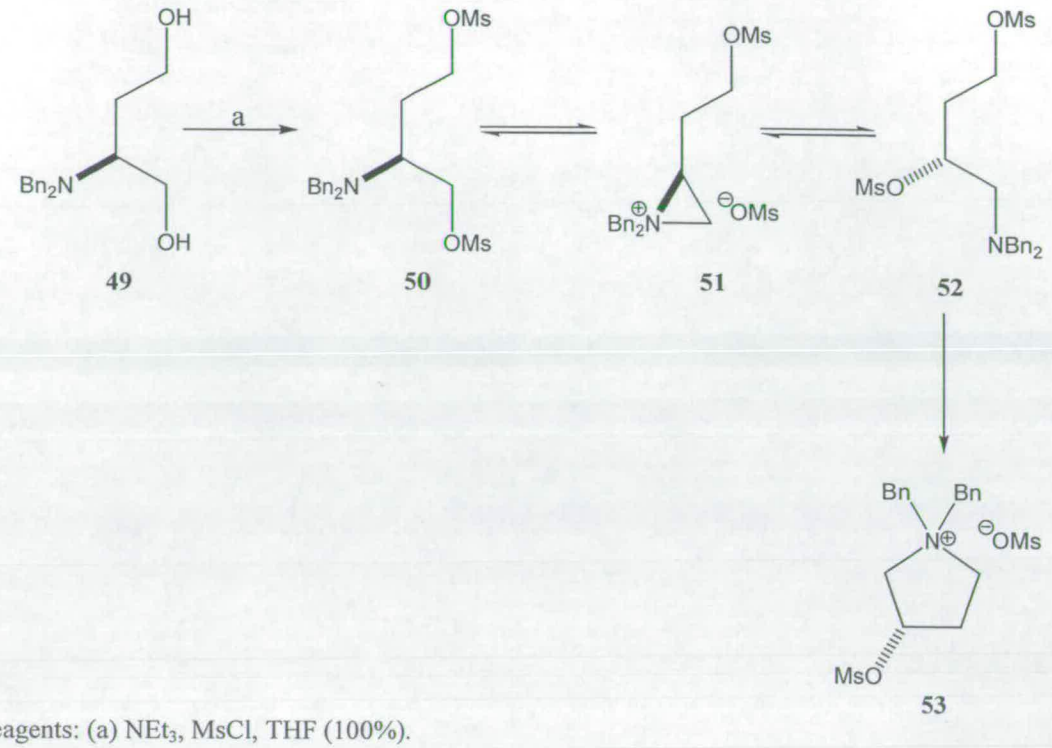
The selective tosylation of the primary alcohol was then attempted by reacting diol **29** with TsCl in the presence of DMAP (**Scheme 18**). However, rather then isolating the protected alcohol **46**, a salt was obtained which we suspected to be **47**.



Reagents: (a) TsCl, DMAP, CH_2Cl_2; (b) Dowex, Cl^- (85%).

Scheme 18

Our suspicions were based on the fact that the salt produced, gave a more polar spot on the t.l.c. plate than the starting material **29**. Our suspicions grew when the distinctive tosylate peaks in the ^1H NMR (δ 2.29 (s), δ 7.83 (d, *J* 8.2 Hz), and δ 7.10 (d, *J* 8.2 Hz)) diminished in size after washing the compound with 1% hydrochloric acid. This suggested to us that the tosylate counter ion was being exchanged for a chloride ion. After consulting with the literature and finding that others had experienced a similar result (**Scheme 19**),[100] we were left in no doubt that what we had isolated was indeed the pyrrolidinium tosylate salt **47**.

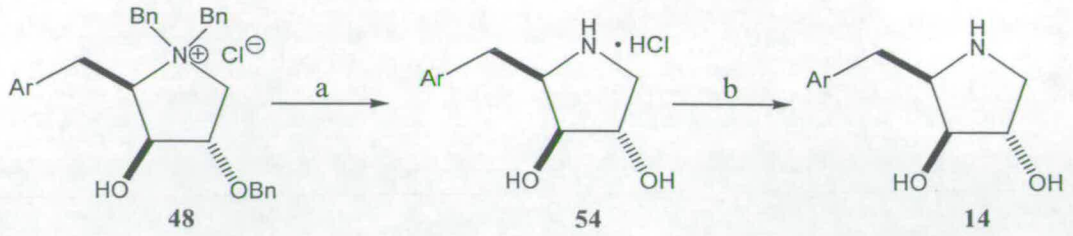


Reagents: (a) NEt_3, MsCl, THF (100%).

Scheme 19

Having obtained salt **47**, we needed to address the fact that it contained a mixture of two counter ions. This problem was solved by passing a solution of the salt through a Dowex column treated with 1% hydrochloric acid. This had the desired effect of exchanging both counter ions for the chloride ion, thereby ensuring that only one salt remained. ^{1}H NMR confirmed this to be the case.

The chloride salt obtained was then hydrogenated in the presence of Pearlman's catalyst to give deacetylanisomycin as its hydrochloride salt. This salt was again subjected to a Dowex column, this time treated with 1 M NaOH, to give deacetylanisomycin as its free base, and in excellent yield (**Scheme 20**).



Reagents: (a) H_2, 20% $Pd(OH)_2$, CH_3OH (100%); (b) Dowex OH^- (100%).

Scheme 20

A comparison of the literature ^1H NMR and optical rotation data with compounds **54** and **14** is shown in **Tables 2** and **3**. The data for the two compounds is clearly in good agreement with that of the literature, confirming the successful synthesis of deacetylanisomycin, in 11 steps and in an overall yield of 40%. The result also suggests that the stereochemistry tentatively assigned to aldol adduct **30** is correct.

Table 2 : A Comparison of NMR Data with Literature Data for Deacetylanisomcyin

	Source	MHz	Solvent	2-H	3-H	4-H	5-H_A	5-H_B	1'-H
Salt	Ref 14[a]	205.1	CD_3OD	3.91	4.02	4.30	3.14	3.66	3.00, 3.20
	54	250	CD_3OD	3.96	4.07	4.34	3.18	3.70	3.04, 3.24
Free Base	Ref 90(v)	200	DMSO-d_6	2.96	3.45 – 3.47	3.82 – 3.85	2.36	3.15	2.52, 2.71
	Ref 90(h)	400	DMSO-d_6	3.26	3.73	4.07	2.64	3.38	2.71, 2.86
	14	250	CD_3OD	3.34	4.16	3.79	3.46	2.71	2.97, 2.80

[a] Data obtained for hydrobromide salt

Table 3 : A Comparison of NMR and Optical Rotation Data with Literature Data for Deacetylanisomcyin

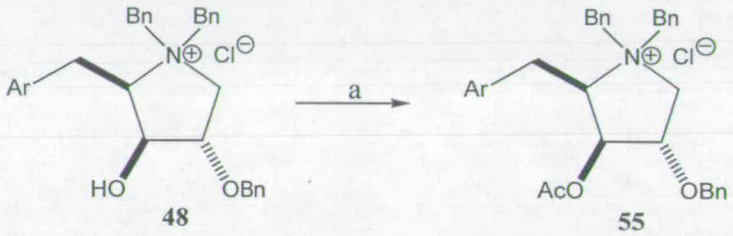
	Source	$J_{2,3}$ (Hz)	$J_{3,4}$ (Hz)	$J_{4,5A}$ (Hz)	$J_{4,5B}$ (Hz)	$J_{5A,5B}$ (Hz)	$J_{2,1'}$ (Hz)	$J_{1'A,1'B}$ (Hz)	$[\alpha]_D$
Salt	Ref 14[a]	2.8	1.5	0	4.4	12.4	8.2, 7.1	14.0	+7.2 (*c* 1.0, CH_3OH)
	54	2.8	1.4	0	4.2	12.5	8.3, 7.1	14.1	+8.1 (*c* 0.6, CH_3OH)
Free Base	Ref 90(v)	3.5	–	2.1	5.5	11.8	7.4, 6.5	13.2	-22.5 (*c* 1.0, CH_3OH)
	Ref 90(h)	3.4	0	2.5	5.9	12.2	7.2, 7.2	13.6	-20 (*c* 1.0, EtOH)
	14	3.8	1.4	2.0	5.7	12.4	8.3, 6.6	13.4	-19.8 (*c* 1.0, CH_3OH)

[a] Data obtained for hydrobromide salt

2.6 Synthesis of Anisomycin

With the synthesis of deacetylanisomycin successfully completed, and the stereochemistry of aldol adduct **30** confirmed, our attentions turned towards completing the synthesis of anisomycin.

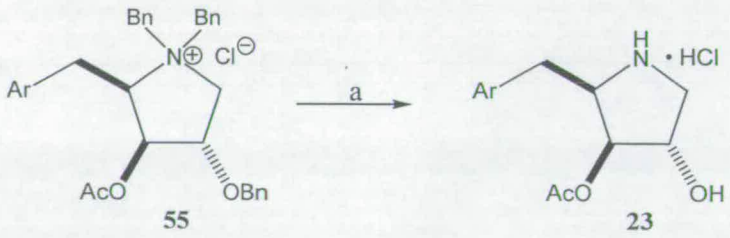
Having obtained the pyrrolidinium salt **48**, we next set about acetylating it. Unfortunately, treatment of the salt with acetic anhydride and DMAP gave another pyrrolidinium salt with a similar R_f value (**Scheme 21**). Consequently, clean separation of the product from the starting material using flash chromatography proved difficult, and only a modest yield of 54% was obtained.



Reagents: (a) Ac_2O, DMAP, CH_2Cl_2 (54%).

Scheme 21

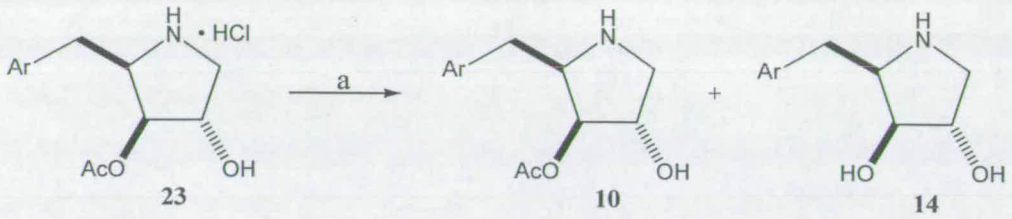
With salt **55** in hand, albeit not very much, we decided to try and globally deprotect it to give anisomycin. Exposure of the salt to a hydrogen atmosphere in the presence of Pearlman's catalyst gave, as anticipated, anisomycin as its hydrochloride salt (**Scheme 22**).



Reagents: (a) H_2, 20% $Pd(OH)_2$, CH_3OH (100%).

Scheme 22

In an attempt to obtain anisomycin as its free base, the salt was passed through a Dowex column treated with 1 M sodium hydroxide. However, rather than obtaining anisomycin as expected, we instead isolated a mixture of both anisomycin and deacetylanisomycin (**Scheme 23**). It was clear therefore, that sodium hydroxide was not only removing the salt, but also hydrolysing the acetate ester to give deacetylanisomycin.

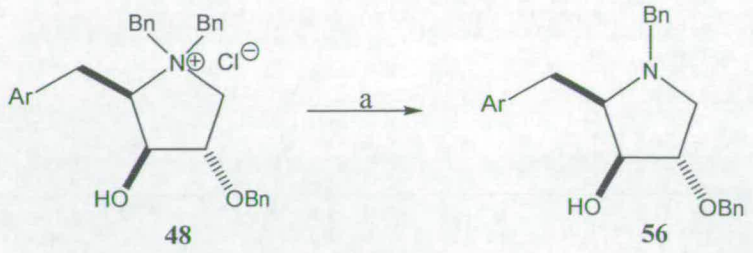


Reagents: (a) Dowex (OH^-).

Scheme 23

With both steps producing their own individual set of problems, we set about looking at a different route to anisomycin. One possible route focused on the selective removal of a benzyl group from the nitrogen centre of the dibenzyl quaternary pyrrolidinium salt **48**. This would free up the salt without affecting our synthetic strategy, allowing us to access anisomycin using the same procedure as before.

Initial attempts focused on hydrogenating the salt using differing lengths of time and various quantities and types of catalyst (**Table 4**). However, none of these attempts proved successful with mono-, di-, and fully-debenzylated material being recovered. It was not until the hydrogenation was carried out under basic conditions that any noticeable selectivity was observed.



48 56

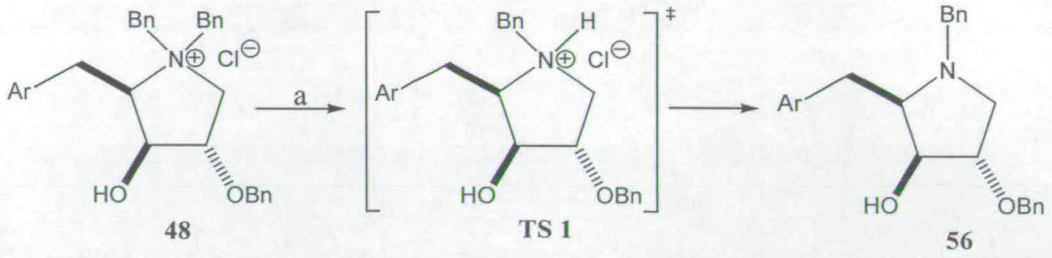
Reagents: (a) see **Table 4**.

Scheme 24

Table 4: Conditions Used in the Selective Monobenzylation of Pyrrolidinium Salt 48

Entry	Reagents/Conditions	Time (Minutes)	Yield
1	20% $Pd(OH)_2/C$ (100% Mass eq.)	25	N/E
2	20% $Pd(OH)_2/C$ (10% Mass eq.)	25	50
3	5% Pd/C (10% Mass eq.) NH_4OAc (0.05 mol eq.)	25	70
4	10% Pd/C (100% Mass eq.) Formic acid/MeOH (1 mol eq.)	18	49
5	5% Pd/C (10% Mass eq.) K_2CO_3 (3 mol eq.)	10	94

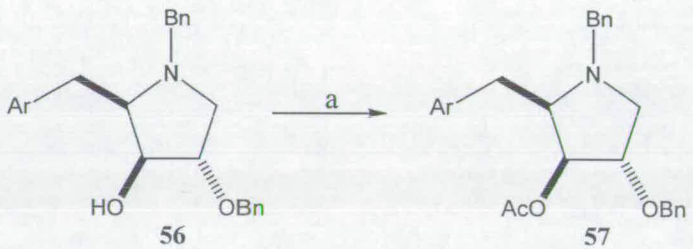
When the salt **48** was hydrogenated in the presence of K_2CO_3, the alcohol **56**, was isolated cleanly and in excellent yield. It was therefore concluded that the positive charge on the nitrogen centre appeared to increase the rate of debenzylation. Consequently, when the reaction was carried out under basic conditions the formation of a free base intermediate after hydrogenylitic removal of the first benzyl protecting group appeared to slow the rate of any further hydrogenation, thereby allowing us to access **56** in excellent yield (**Scheme 25**).



Reagents: (a) H_2, 5% Pd/C, K_2CO_3.

Scheme 25

Having successfully obtained **56** we again set about repeating the final two steps of the synthesis. This time treatment of **56** with Ac_2O, NEt_3, and DMAP proceeded as expected, and the product **57** was isolated in 92% yield (**Scheme 26**). The global deprotection of **57** to obtain anisomycin was then attempted.

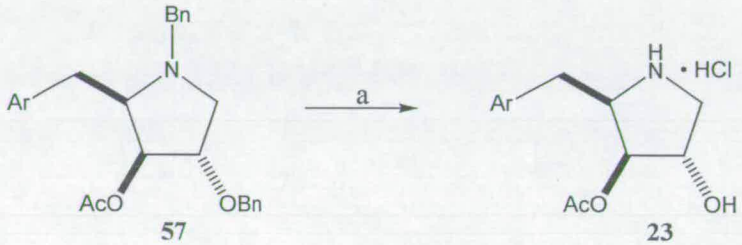


Reagents: (a) Ac_2O, NEt_3, CH_2Cl_2 (92%).

Scheme 26

When **57** was hydrogenated in the presence of Pearlman's catalyst, anisomycin was isolated as a mixture of its salt and its free base. The formation of the salt was attributed to the Celite used when filtering the reaction mixture. It became apparent after some time that the Celite obtained from various commercial sources is pre-washed with acid. Consequently, when mixing amines and Celite together in a solvent such as methanol, there is a strong possibility of partial salt formation.

To counteract this problem it was decided to repeat the reaction with 2 equivalents of 1 M hydrochloric acid dissolved up in the reaction mixture. It was hoped that anisomycin would form its hydrochloride salt during the reaction, thereby eliminating the problem of partial salt formation by Celite at the end of the reaction. Thus, as predicted, exposure of **57** to a hydrogen atmosphere in the presence of methanol and 1 M hydrochloric acid, gave after stirring, anisomycin as its hydrochloride salt in excellent yield (**Scheme 27**).



Reagents: (a) H_2, 20% $Pd(OH)_2/C$, 1 M HCl/Et_2O, CH_3OH (100%).

Scheme 27

A comparison of the literature ^1H NMR and optical rotation data with **23** is shown in **Tables 5**, **6**, and **7**. The data for **23** is in clear agreement with that of the literature. This therefore suggests that we have, as thought, successfully synthesised anisomycin as its hydrochloride salt, in 13 steps and with a 35% overall yield. The result again reconfirms our earlier assignment of stereochemistry to aldol adduct **30**.

Table 5 : A Comparison of Literature NMR Chemical Shift Data with 23

Source	MHz	Solvent	2-H	3-H	4-H	5-H_A	5-H_B	1'-H	Other
Ref 14	205.1	CD_3OD	4.21	5.10	4.40	3.24	3.65	3.02, 3.13	2.23, 3.82, 6.96, 7.28
23	250	CD_3OD	4.23 - 4.15	5.08	4.38	3.22	3.63	2.99, 3.11	2.21, 3.81, 6.95, 7.26

Table 6 : A Comparison of Literature NMR Multiplicity Data with 23

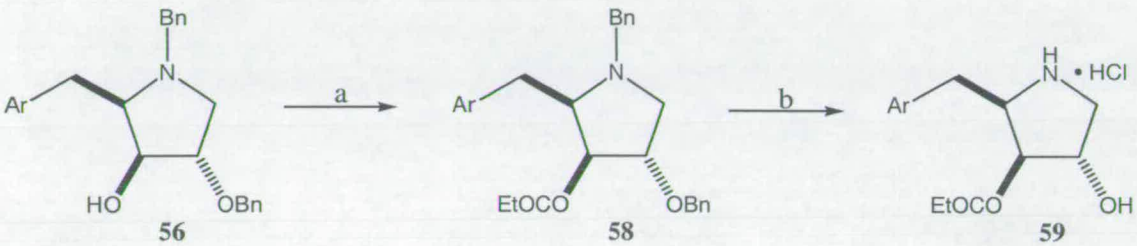
Source	$J_{2,3}$ (Hz)	$J_{3,4}$ (Hz)	$J_{4,5A}$ (Hz)	$J_{4,5B}$ (Hz)	$J_{5A,5B}$ (Hz)	$J_{2,1'}$ (Hz)	$J_{1'A,1'B}$ (Hz)	J_{Ar} (Hz)
Ref 14	3.4	0	0	4.5	12.7	8.7 6.9	14.2	8.5
23	3.0	0	0	4.3	12.8	8.8, 6.7	14.3	8.6

Table 7 : A Comparison of Literature Optical Rotation Data with 23

Source	$[\alpha]_D$
Ref 14	+4.2 (*c* 0.51, CH_3OH)
23	+4.0 (*c* 0.28, CH_3OH)
Ref 1	+3.9 (*c* 1.0, CH_3OH)
Ref 90(d)	+3.5 (*c* 1.0, CH_3OH)

2.7 Synthesis of 3097-B1

Having successfully synthesised anisomycin our attentions turned to synthesising 3097-B1 (**59**), another natural product also isolated from the fermentation broths of *Streptomyces* strain SA3097. This natural product differs only slightly from anisomycin in having a propionate ester at the C(3) position rather than an acetate group. It was therefore envisaged that treatment of **56** with propionic anhydride as opposed to acetic anhydride would enable us to obtain, after



deprotection, 3097-B1.

Reagents: (a) $(EtCO)_2O$, NEt_3, CH_2Cl_2 (89%); (b) H_2, 20% $Pd(OH)_2/C$, 1 M HCl/Et_2O, CH_3OH (100%).

Scheme 28

As anticipated, treatment of **56** with propionoic anhydride proceeded smoothly to give **58** in 89% yield. Deprotection of **58**, employing the same conditions as that for anisomycin, then gave 3097-B1 as its hydrochloride salt, in quantitative yield (**Scheme 28**).

2.8 Summary of Chapter 2

In this chapter, the successful synthesis of both anisomycin and deacetylanisomycin has been described. The chapter began with the analysis of several previous syntheses of anisomycin, and it was noted that many of these syntheses suffered from a series of protection and deprotection steps in the latter stages. Bearing this in mind, we put forward our own retrosynthesis.

In the next section, the synthesis of a tyrosine derived aldehyde was presented. Its employment in a 'mismatched' asymmetric boron mediated aldol reaction proved lucrative in setting up the stereochemistry required for both natural products. Consequently, it enabled us to synthesise both anisomycin and deacetylanisomycin from D-tyrosine in 13 steps, 35% overall yield and 11 steps 40% overall yield respectively.

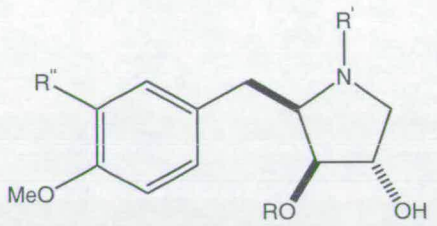
Chapter 3 : The Biological Evaluation of Some Anisomycin Derivatives

3.1 A Review of Anisomycin SAR Studies

Since the mid 1960's several groups have been interested in the study of various analogues of anisomycin. Many of these early studies focused on anisomycin's behaviour as an antibiotic and sought to produce analogues that would improve its biological activity. Following a lull during the 1980's, interest in anisomycin was again sparked in 1993 when it was discovered that anisomycin gave high anti-tumour activity *in vitro*. This interest has lasted for a period of ten years during which time it has also become apparent that anisomycin is able to act as a signalling agonist for the stress-activated, mitogen-activated protein kinase pathways, JNK and p38, in mammalian cells. It is therefore somewhat surprising, that at the time of writing, a structure activity relationship (SAR) study involving analogues of anisomycin and the JNK or p38 MAP kinase pathways has not been carried out. Similarly, it is also surprising, given that anisomycin bears close structural homology with the iminosugars, that neither anisomycin nor its family of naturally occurring analogues have been studied as potential glycosidase inhibitors.

3.1.1 Evaluation of Antibiotic Activity

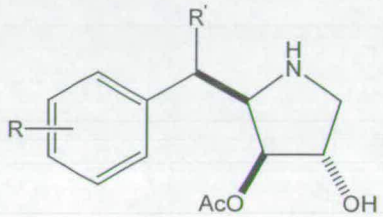
In 1967 Grollman[45] studied the activity of several analogues of anisomycin in cell-free protein-synthesising systems, from both rabbit reticulocytes and *Sacharomyces fragilis* (**Figure 19**). He found that all the analogues tested had less than 1% of the activity of anisomycin. From his research he concluded that the basic pyrrolidine ring is important for the activity of anisomycin, since either acetylation of the nitrogen atom or deacetylation at the C(3)-*O* position renders the molecule inactive. Similar results were also observed when the *p*-methoxyphenyl group was brominated at the *meta* position suggesting that only a *para* substituted phenyl ring would be tolerated by the active site of the enzyme. These results led Grollman[101] to propose that the essential structural features required for this biological activity are a secondary amine group adjacent to an asymmetric carbon (*R* configuration) linked *via* a methylene to a sterically unhindered six-membered ring.



Compound	R	R'	R''	IC_{50} [μM]			
				Rabbit reticulocytes		*S. fragilis*	
				Haemoglobin synthesis in intact cells	*Haemoglobin synthesis in cell free system*	Growth	Polyphenylalanine synthesis in cell free system
10	Ac	H	H	0.05	8	7	8
14	H	H	H	50	>1000	300	>1000
60	Ac	Ac	H	100	>1000	>1000	>1000
61	Ac	H	Br	7	>1000	500	>1000
62	Ac	$(CH_3)_2$	H	100	>1000	>1000	>1000

Figure 19 : Protein Synthesis Inhibition by Anisomycin Analogues

Advances in synthetic chemistry during the 1970's allowed Hall and co-workers[102] to synthesise a more diverse range of analogues from pyrrole-2-carboxaldehyde. These analogues were tested alongside anisomycin for antiprotozoal activity on *Trichomonas vaginalis, Trichomonas foetus, and Entamoeba histolytica* (**Figure 20**).[103] It was found that their activity against protozoa and fungi was restricted primarily to anisomycin and the *p*-methylbenzyl and benzyl analogues. Interestingly, as the methoxy group changed from the *para* to the *ortho* position on the aromatic ring the biological activity dropped considerably, thereby adding weight to the previous observations of Grollman.



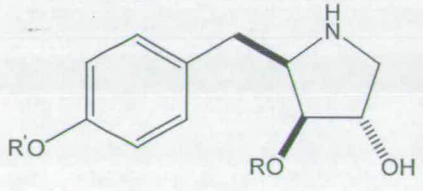
Compound	R	R'	IC_{95} [μM]		
			Trichomonas vaginalis	*Trichomonas foetus*	*Entamoeba histolytica*
(-) 10	*p*-OMe	H	1.9	3.8	3.8
(±) 10	*p*-OMe	H	3.8	7.6	7.6
(±) 63	*p*-Me	H	16.1	16.1	8.0
(±) 64	H	H	17.0	68.1	8.5
(±) 65	*m*-OMe	H	60.4	60.4	60.4
(±) 66	*o*-OMe	H	-	-	-
(±) 67	*p*-OMe	Me	-	-	-
(±) 68	*p*-OMe	Ph	-	-	-

Figure 20 : Antiprotozoal Activity of Anisomycin Analogues

3.1.2 Evaluation of Anti-Tumor Activity

In 1993 Kameyama[2] isolated two new analogues of anisomycin, along with the rest of the known family of naturally occurring analogues, from the fermentation broths of *Streptomyces*, strain SA3097. These new analogues differed from anisomycin and demethylanisomycin **71**, in that they both had a propionate ester at the C(3)-*O* position rather than an acetate group.

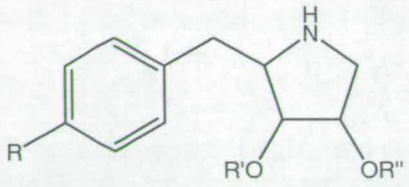
The biological activity of the analogues was tested on two human tumor cell lines, LU99 and MCF7 (**Figure 21**). Kameyama observed that changing between acetate and propionate esters on the ring seemed to have little effect on the cytotoxicity. It was also noted that demethylation at the aromatic ring also had little effect on activity as long as the pyrrolidine ring preserved its acyl group. However, in contrast, it was found that deacylation of the pyrrolidine ring resulted in a considerable reduction of activity and additional demethylation resulted in a complete loss of activity.



Compound	R	R'	IC_{50}[nM]	
			Tumor Cell Line	
			LU99	MCF7
69	EtCO	Me	65	90
70	EtCO	H	85	129
10	Ac	Me	50	95
71	Ac	H	82	74
14	H	Me	23	34
72	H	H	153000	175000

Figure 21 : Tumor Cell Line Inhibition by Anisomycin Analogues

Recently, Jäger[14] synthesised some novel analogues of anisomycin from diethyl L-tartrate. Jäger tested these compounds alongside some of the naturally occurring analogues for *in vitro* cytotoxic activity against human cell lines KB, HBL 100 RAS A, and HBL 100 (as a reference, non tumour cell line). From his results he observed that the benzyl analogue **20**, showed only a slight decrease in cytotoxicity against the cell lines (**Figure 22**). Acetylation of both hydroxyl groups at positions 3 and 4, **77** again led to only a slight decrease in cytotoxicity. However, deacetylation of the pyrrolidine ring led to a strong reduction of activity and additional removal of the methoxy group gave rise to a complete loss of cytotoxicity. Interestingly, the *N,O*-dibenzyl compounds and the 3-*O*-benzoyl analogue give rise to approximately the same decrease in cytotoxicity as deacetylanisomycin. However, analogues which had different stereochemistry to that of anisomycin, were found to be inactive towards the cell lines.



Compound	Stereochemistry	R	R'	R''	IC_{50}[μM]		
					KB	HBL 100 RASA	HBL 100
73[a]	2*R*,3*S*,4*S*	H	Bn	H	14	51	79
20[a]	2*R*,3*S*,4*S*	OMe	Bn	H	20	45	55
74	2*R*,3*S*,4*S*	H	H	H	-	-	-
14	2*R*,3*S*,4*S*	OMe	H	H	19	50	29
59	2*R*,3*S*,4*S*	H	Ac	H	0.40	0.094	0.079
10	2*R*,3*S*,4*S*	OMe	Ac	H	0.01	0.05	0.05
75	2*R*,3*S*,4*S*	H	Bz	H	-	-	-
76	2*R*,3*S*,4*S*	OMe	Bz	H	24	9	18
77	2*R*,3*S*,4*S*	OMe	Ac	Ac	0.1	0.3	0.2
78	2*S*,3*S*,4*S*	H	H	H	-	-	-
79	2*S*,3*S*,4*S*	OMe	H	H	-	-	-
80	2*S*,3*R*,4*S*	OMe	H	H	-	-	-
81	2*S*,3*R*,4*S*	OMe	H	Bn	-	-	-

[a] Contains benzylated nitrogen centre.

Figure 22 : Tumor Cell Line Inhibition by Anisomycin Analogues

3.2 Synthesis of Anisomycin Derivatives

The review of anisomycin based SAR studies suggests that the ester group at the C(3)-*O* position is crucial for biological activity (**Figure 23**). The review also indicates that although acetate protection of the secondary amine is detrimental to anisomycin's activity, acetate protection of the C(4) hydroxyl group causes only a slight decrease in its biological activity. It was therefore proposed that the enzyme's active site might be tolerant to changes to the substituent at this position and consequently, we decided to target the C(4) position of anisomycin to produce our range of analogues.

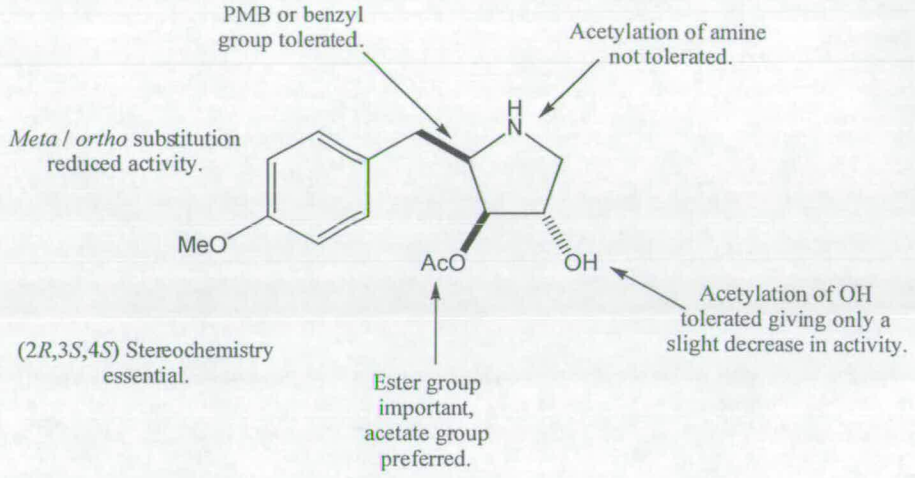


Figure 23 : A Summary of SAR Studies on Anisomycin

As highlighted, the presence of an ester group at the C(3)-*O* position is crucial for anisomycin's biological activity. It was proposed that the ester's role might be to decrease the polarity of the iminosugar in order to enable it to cross cell membranes. It was also proposed that once inside the cell, the ester group might be removed by hydrolysis to give deacetylanisomycin and that this might therefore be the active form of the drug.

Given these considerations, we decided to focus on synthesising the C(4)-Me **82** and C(4)-H **84** analogues of anisomycin along with their deacetyl derivatives **83** and **85** (**Figure 24**). It was hoped that biological systems might absorb our lipophilic analogues better than anisomycin. It was also proposed that if deacetylanisomycin was indeed the active form of the drug, and its biological activity was being hampered by its polarity, then we might expect to see some form of activity from our less polar deacetyl derivatives.

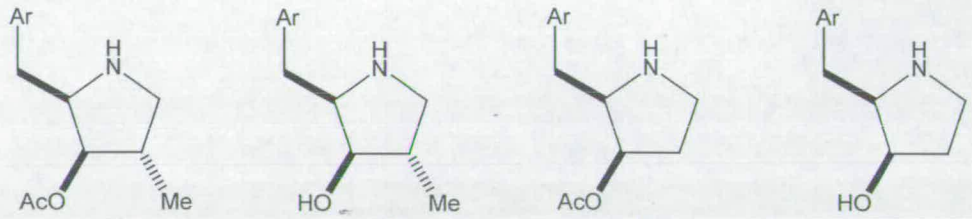


Figure 24 : Synthetic Analogues of Anisomycin

Previous work within the Hulme group had already allowed us to successfully synthesise the C(4)-Me derivative of DAB-1[104] **86** and a derivative of the natural product CYB-3[105] **87** (**Figure 25**). We were therefore optimistic that similar approaches would enable us to access our anisomycin analogues.

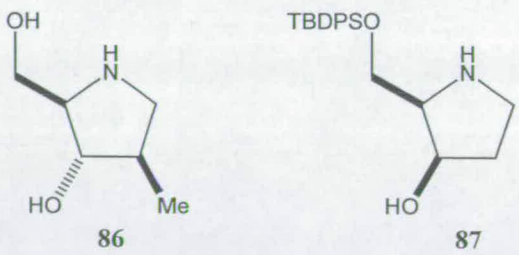


Figure 25 : Synthetic Analogues of DAB-1 and CYB-3

3.2.1 Synthesis of C(4)-Me Analogue

Our retrosynthetic analysis for the C(4)-Me derivative of anisomycin is shown in **Figure 26**. The retrosynthesis is based on similar methodology to that used in the synthesis of anisomycin. Thus we envisaged that the aldol reaction between aldehyde **31** and the chiral propionate equivalent **91** would give aldol adduct **90** as a single diastereomer in an analogous manner to that observed for the glycolate aldol reaction. From here on, we believed that the chemistry undertaken for the synthesis of anisomycin would be directly applicable to its C(4)-Me analogue.

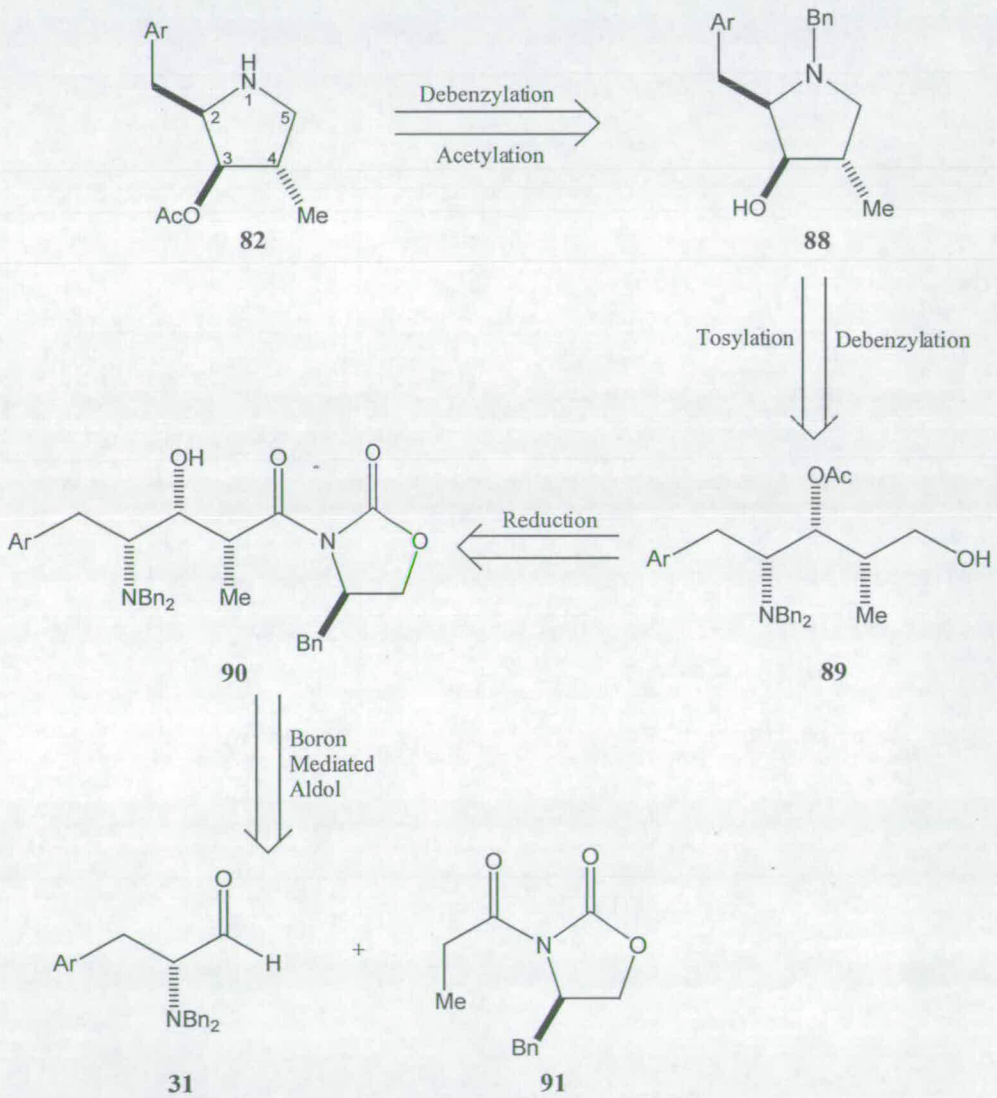
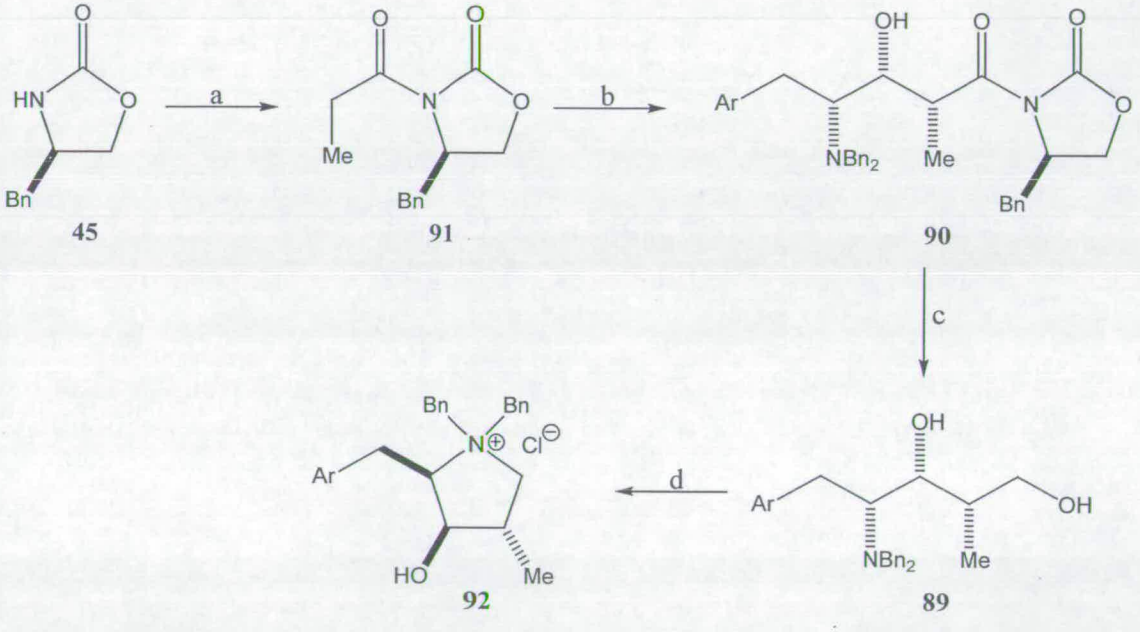


Figure 26 : The Retrosynthesis of the C(4)-Me Analogue

The chiral propionate equivalent was synthesised from oxazoldin-2-one **45** using the same protocol as that used for the synthesis of **32**. Thus, reaction of **45** with *n*-butyllithium gave the lithiated oxazolidin-2-one, which on condensation with freshly distilled propionyl chloride gave **91** in excellent yield (**Scheme 29**).

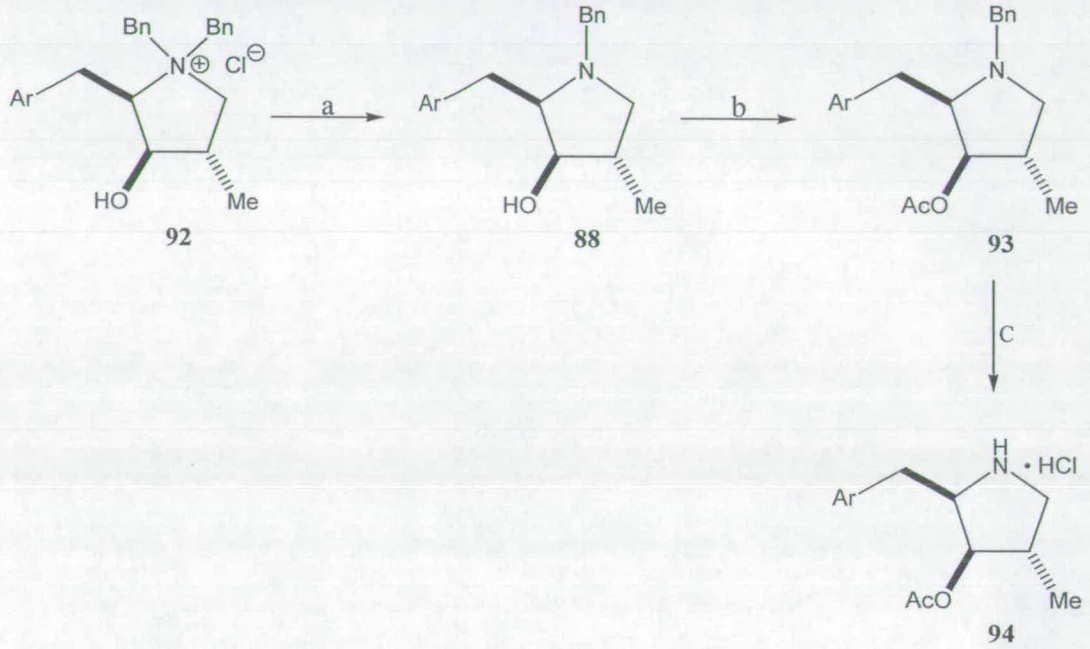
The aldol reaction between imide **91** and aldehyde **31** proceeded as anticipated to give aldol adduct **90** as a single diastereomer and in good yield. The reduction of **90** with lithium borohydride gave diol **89** (82%) and the auxiliary was recovered in 62% yield. Treatment of the diol with TsCl selectively protected the primary alcohol causing it to cyclise to give the pyrrolidinium tosylate salt. Treatment of this salt with Dowex resin, pretreated with 1% HCl, gave the chloride salt in good yield without any ditosylated material being observed.



Reagents: (a) BuLi, THF, C_2H_5COCl (90%); (b) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) **31** (87%); (c) $LiBH_4$, CH_3OH, THF (82%); (d) (i) TsCl, DMAP, CH_2Cl_2; (ii) Dowex, Cl^- (83%).

Scheme 29

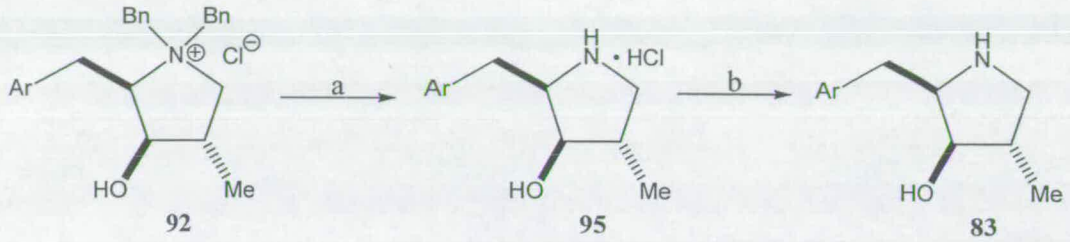
Having successfully obtained reaction conditions to mono-deprotect the pyrrolidinium salt during the synthesis of anisomycin, we were confident that similar conditions would enable us to selectively deprotect **92**. This proved to be the case with hydrogenolysis of the pyrrolidinium salt in the presence of 5% Pd/C and potassium carbonate giving the mono protected amine in good yield. Finally, acetylation of the secondary alcohol and deprotection of the amine functionality, employing the same protocol as that required for anisomycin, gave as expected the C(4)-Me derivative as its hydrochloride salt in quantitative yield (**Scheme 30**).



Reagents: (a) H_2, 5% Pd/C, K_2CO_3, CH_3OH (93%); (b) Ac_2O, NEt_3, CH_2Cl_2 (82%); (c) H_2, 20% $Pd(OH)_2/C$, 1 M HCl/Et_2O, CH_3OH (100%).

Scheme 30

In order to obtain the C(4)-Me deacetyl derivative the dibenzyl salt **92** was hydrogenated in the presence of Pearlman's catalyst to give the deacetyl derivative as its hydrochloride salt. As anticipated, treatment of the salt with Dowex resin, pretreated with 1 M sodium hydroxide, gave the C(4)-Me deacetyl derivative as its free base in excellent yield (**Scheme 31**).



Reagents: (a) H_2, 20% $Pd(OH)_2/C$, CH_3OH (100%); (b) Dowex OH^- (100%).

Scheme 31

3.2.2 Synthesis of the C(4)-H Analogue

The successful synthesis of the two methyl analogues was achieved by swapping the glycolate aldol coupling partner **32** for the chiral propionate equivalent **91**. However our next target, the C(4)-H derivative **84**, required the complete removal of the substituent at the C(4) position and in order to address this problem, we developed the following retrosynthesis (**Figure 27**).

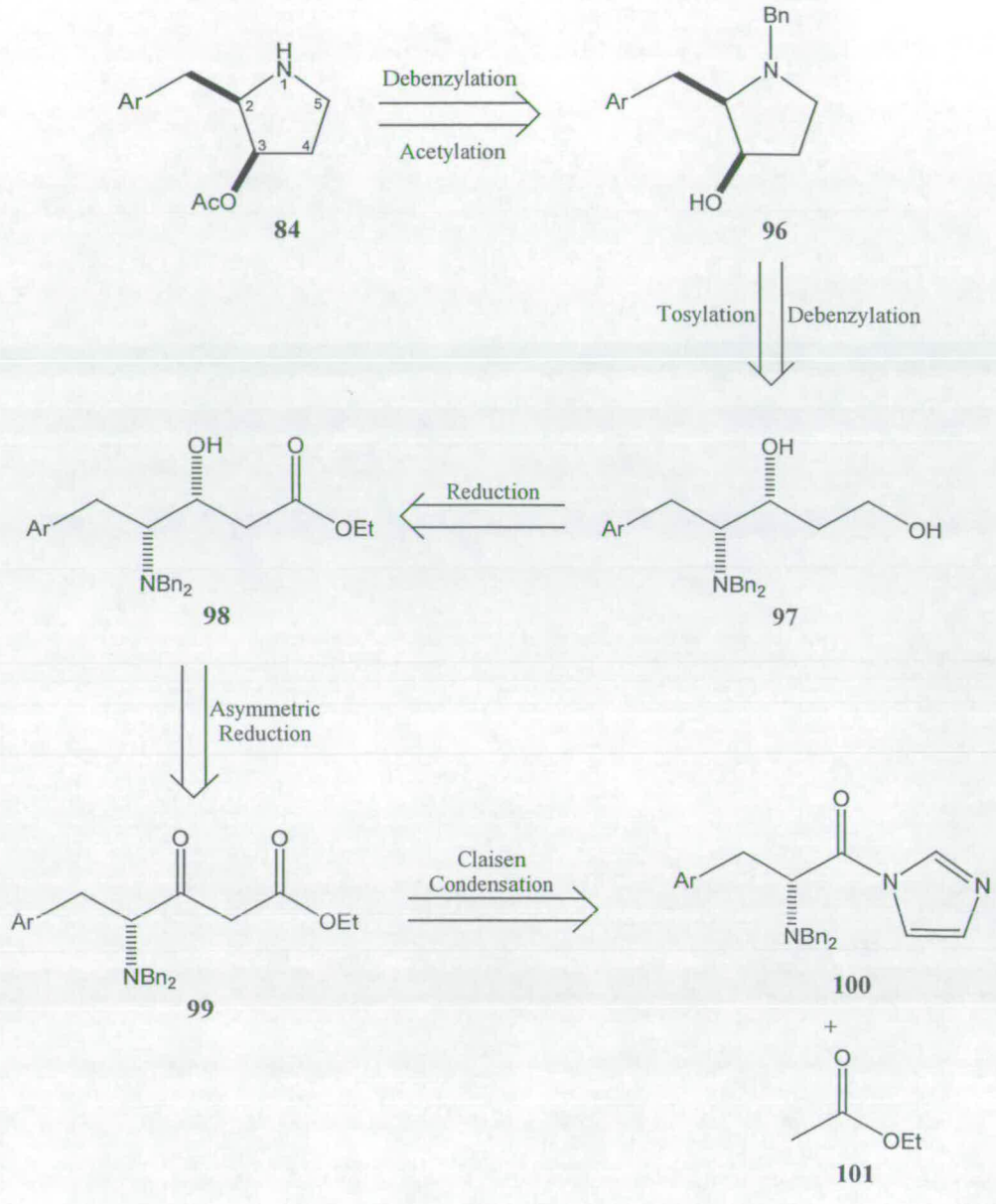
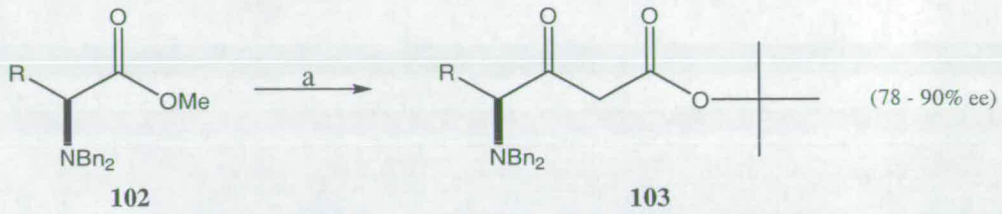


Figure 27 : The Retrosynthesis of the C(4)-H Analogue

It was anticipated that the Claisen condensation between imidazolide **100** and the lithium enolate of ethyl acetate would allow us to obtain the β-keto ester **99**. It was then hoped that the asymmetric reduction of **99** would produce alcohol **98** which upon further reduction, would give diol **97**. From here on we believed that the chemistry undertaken in the synthesis of anisomycin, would allow us to access the C(4)-H derivative.

Our decision to convert the methyl ester to its imidazolide prior to the Claisen condensation was based on findings reported by Hoffman.[106] He found that β-keto esters formed *via* the reaction of α-amino esters with lithio *tert*-butyl acetate were subject to a considerable loss of enantiopurity (**Scheme 32**).



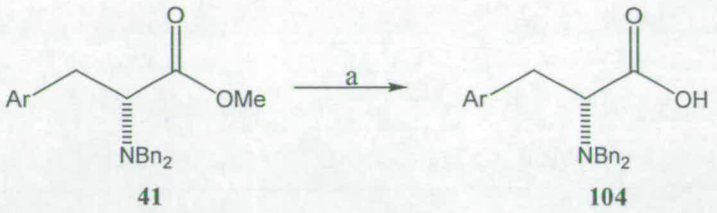
102 **103**

Reagents: (a) $CH_2=C(O^-Li^+)O^tBu$, THF.

Scheme 32

Similar studies within the Hulme group also confirmed this to be the case. However, the conversion of serine and phenylalanine derived esters to their more reactive imidazolides prior to their employment in the Claisen condensation not only reduced racemisation, but eliminated the problem completely in the case of phenylalanine.[107] We were therefore optimistic that conversion of our tyrosine derived ester to its imidazolide would also reduce racemisation in the Claisen condensation.

The type of base used to hydrolyse the methyl esters was also found to be critical in maintaining the enantiopurity of the substrate. Studies within the Hulme group found that when lithium hydroxide was employed as the base it gave the highest yields and the least racemisation.[107] Consequently, methyl ester **41** was treated with lithium hydroxide to give the acid **104** as a single spot and in good yield (**Scheme 33**). This was fortunate as attempts to purify the acid by column chromatography proved problematic and often resulted in low yields of the acid being recovered. It was therefore decided to use the acid crude in subsequent steps.

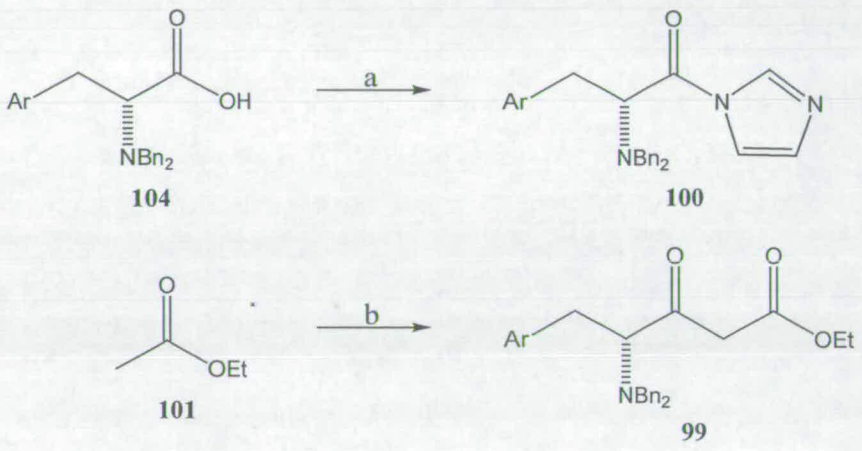


41 104

Reagents: (a) LiOH, THF/H_2O (95%).

Scheme 33

Thus, crude acid **104** was treated with *N,N*-carbonyldiimidazole to form the imidazolide **100**, which without isolation, was then reacted with the lithio ethyl acetate to give the β-keto ester **99** in good yield (**Scheme 34**).



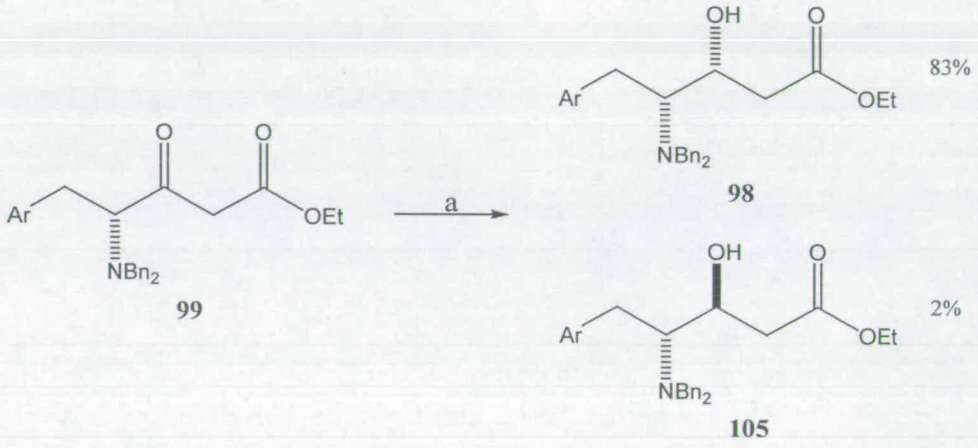
104 100 101 99

Reagents: (a) CDI, THF (b) (i) LiHMDS; (ii) **100** (82%).

Scheme 34

Previous studies within the Hulme group indicated that sodium cyanoborohydride was the reagent of choice for the reduction of our β-keto esters.[105] It was found that this reducing agent gave the corresponding β-hydroxy ester in high yield and with excellent diastereoselectivity. Other reducing agents, in contrast, were found to give lower diastereoselectivities and, in the case of sodium borohydride, resulted in the reduction of the ester functionality.[107]

Treatment of the β-keto ester **99** with sodium cyanoborohydride gave, as anticipated, alcohols **98** and **105** in 83% and 2% yield respectively with no over reduction of the ester moiety (**Scheme 35**). From previous studies, we were optimistic that the major diastereomer had the *syn* stereochemistry, and consequently we assigned the β-hydroxy esters the structures shown.



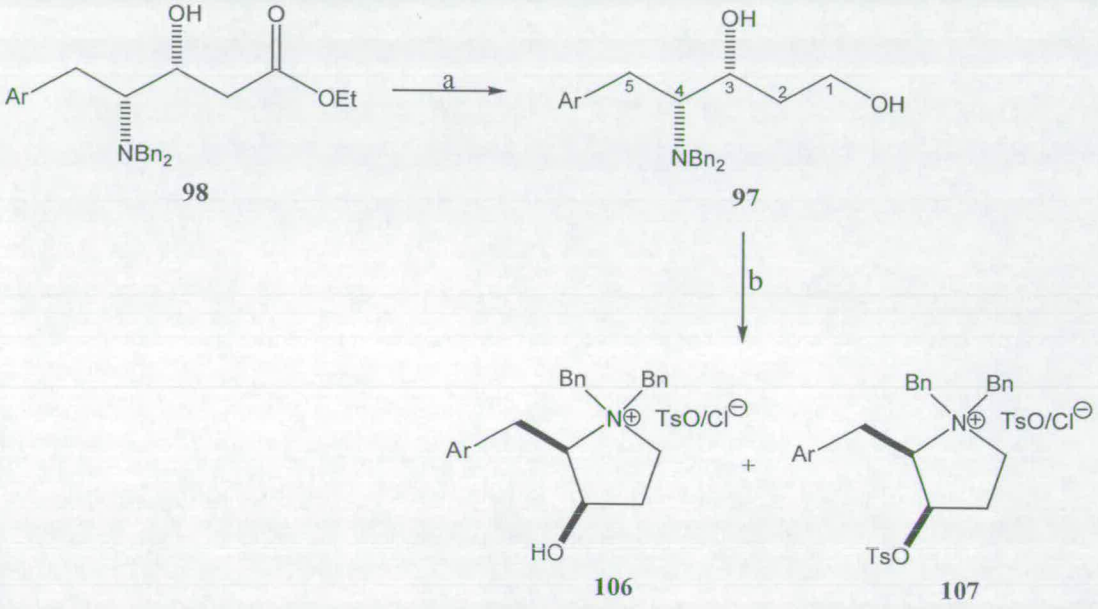
Reagents: (a) $NaCNBH_3$, CH_3OH, AcOH, Et_2O (85%).

Scheme 35

We were concerned about the enatiopurity of ester **98**. This concern was borne from the ease with which racemisation had occurred during a similar synthesis employing a serine derived methyl ester. Therefore, having obtained a moderately polar substrate that we were confident would separate from its enantiomer using reverse phase chiral HPLC, we began to set about determining its enantiopurity.

A racemic synthesis of the β-hydroxy ester **98** was carried out. The racemate obtained was analysed using chiral HPLC (Chiracel OD-H column; solvent 5% propan-2-ol in hexane) in order to optimise column conditions and ensure good baseline peak separation. The single enantiomer was then analysed in the same manner, and reassuringly, showed that no appreciable racemisation had occurred. (material >98% ee, **Appendix B**)

Having successfully determined the optical purity of the β-hydroxy ester it was readily reduced with lithium aluminium hydride to give diol **97** (**Scheme 36**). Disappointingly, protection of the primary alcohol with TsCl failed to give a single product and instead gave a mixture of the pyrrolidinium salts **106** and **107**. It was clear therefore, that in the absence of a substituent at the C(2) position, the sulfonyl chloride was able to attack the secondary alcohol.

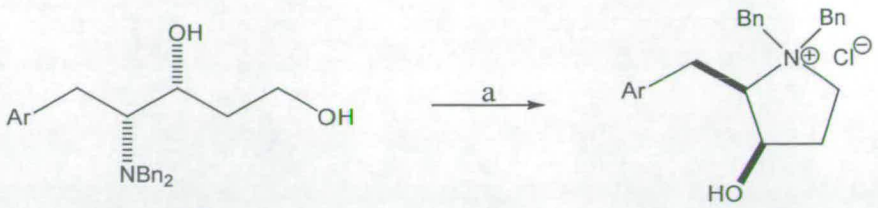


Reagents: (a) $LiAlH_4$, THF (95%); (b) TsCl, DMAP, CH_2Cl_2 (~84%).

Scheme 36

In order to counteract this problem the amount of sulfonyl chloride in the reaction mixture was reduced from 3 to 1 equivalents, and the more sterically demanding triisopropylbenzene sulfonyl chloride (TIBSCl) was employed. It was hoped that the combined steric bulk of this reagent with the *N,N*-dibenzyl group might restrict the reactivity of the sulfonyl chloride to the primary alcohol.

This was found to be the case with TIBSCl and diol **97** reacting to give a single pyrrolidinium salt. The salt produced was then converted to its chloride salt **108** using Dowex resin pretreated with 1% HCl (**Scheme 37**).

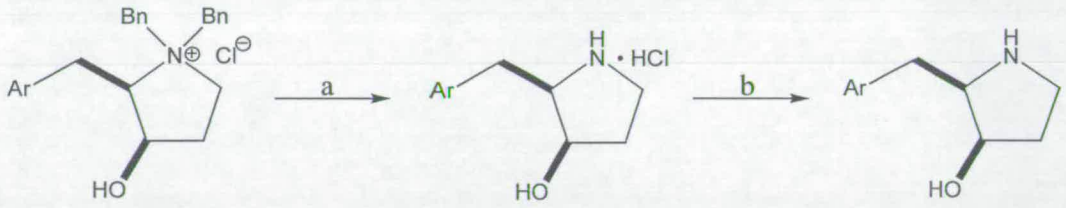


97 108

Reagents: (a) (i) TIBSCl, DMAP, CH_2Cl_2; (ii) Dowex Cl^- (85%).

Scheme 37

In an attempt to obtain the deacetyl derivative and confirm its stereochemistry, the dibenzyl salt was hydrogenated in the presence of Pearlman's catalyst to give the deacetyl derivative **109** as its hydrochloride salt. The salt was then treated with Dowex resin (OH^-) to give the C(4)-H deacetyl derivative **85** as its free base in excellent yield (**Scheme 38**).



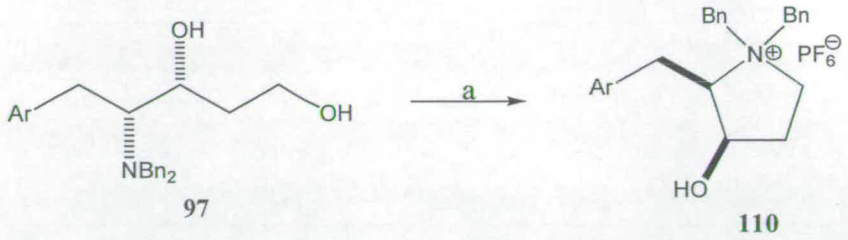
108 109 85

Reagents: (a) H_2, 20% $Pd(OH)_2/C$, CH_3OH (95%); (b) Dowex OH^- (100%).

Scheme 38

In order to confirm the relative stereochemistry of the C(4)-H derivative, a crystal of its hydrochloride salt was grown and submitted for X-ray analysis. Unfortunately, the crystal failed to give an adequate diffraction pattern, and hence its stereochemistry could not be obtained.

It was proposed that the hexaflurophosphate salt of the C(4)-H derivative might produce a better crystal for X-ray analysis due to its relatively larger size. Consequently, the dibenzyl salt was resynthesised and readily converted to its hexafluorophosphate salt **110** using Dowex resin treated with 1% HPF_6 (**Scheme 39**). Surprisingly, the product obtained was a white crystalline solid, which readily crystallised from methanol, to provide a suitable crystal for X-ray analysis (**Figure 28**).



97 110

Reagents: (a) (i) TIBSCl, DMAP, CH_2Cl_2; (ii) Dowex PF_6^- (70%).

Scheme 39

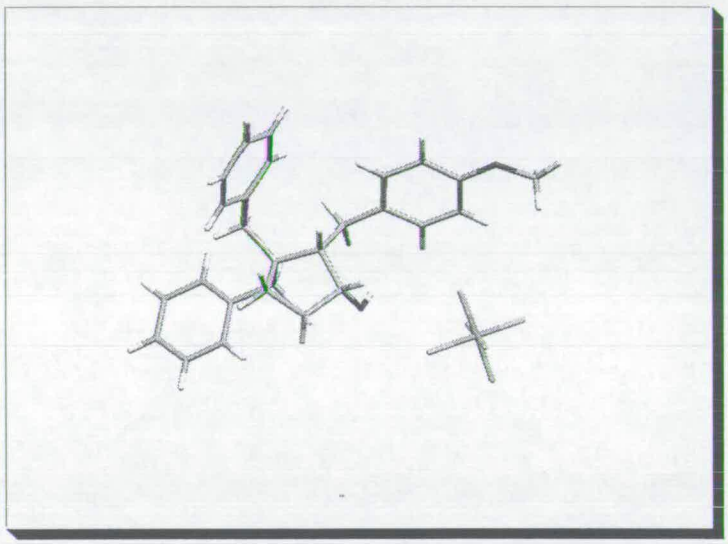
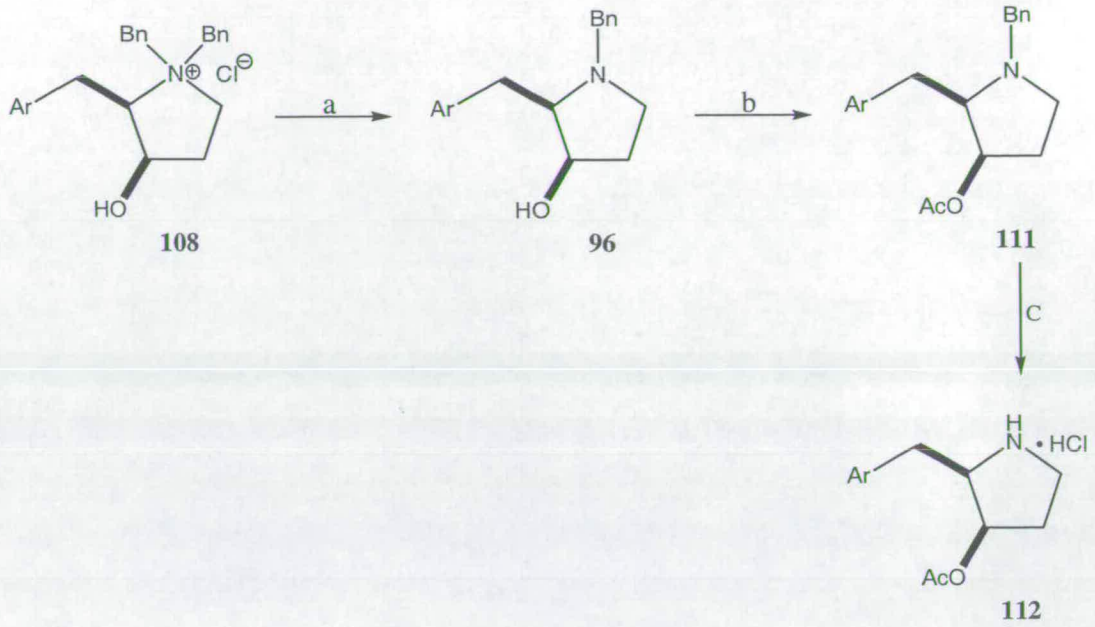


Figure 28 : X-Ray Crystal Structure of 110

The resulting X-ray crystal structure confirms that the stereochemistry, tentatively assigned after the reduction of the β-keto ester, was in fact correct. The crystal structure also shows **110** to be a dibenzyl pyrrolidinium salt and therefore suggests that salts **48** and **92**, produced during the synthesis of anisomycin and its methyl derivative, are also likely to be dibenzyl pyrrolidinium salts.

In an attempt to complete the synthesis of the C(4)-H derivative, the dibenzyl chloride salt **108** was hydrogenated in the presence of 5% Pd/C and potassium carbonate to give the mono protected amine **96**. The secondary alcohol was then acetylated using acetic anhydride and the nitrogen debenzylated in the presence of 1 M HCl to give the C(4)-H derivative as its hydrochloride salt in quantitative yield (**Scheme 40**).



108 96 111 112

Reagents: (a) H_2, 5% Pd/C, K_2CO_3 (80%); (b) Ac_2O, NEt_3, CH_2Cl_2 (87%); (c) H_2, 20% $Pd(OH)_2/C$, 1 M HCl/Et_2O, CH_3OH (100%).

Scheme 40

3.3 Biological Evaluation of Anisomycin and its Derivatives

Anisomycin and its synthetic derivatives were tested *in vitro* against almond β-glucosidase, yeast α-glucosidase, *E. coli* β-galactosidase and fungal α-arabinofuranosidase for glycosidase inhibition but were found to be inactive in all cases.[108]

3.3.1 A Surface Activity Relationship (SAR) Study

A structure activity relationship study was carried out on the C(4)-H and C(4)-Me derivatives by testing them for activity against the p38 and JNK kinase signalling pathways.[ϕ] This was achieved by stimulating RAW macrophages at 'sub-inhibitory' concentrations (30 μmol/ml) with the analogues and assessing their activation, using immunoblot assays, against phosphorylated c-Jun and MAPKAP-K2 (**Figure 29 & 30**).

[ϕ] The SAR study was conducted in Professor Cohen's laboratories at the University of Dundee.

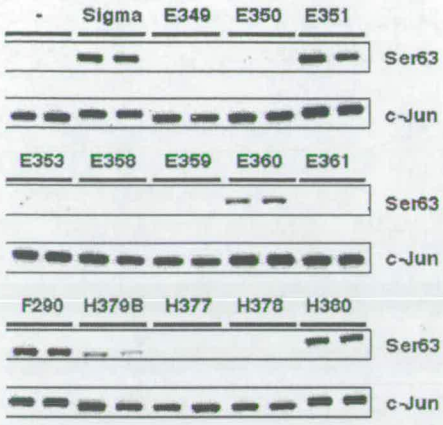


Figure 29 : Effects of Anisomycin and its Derivatives on the Ser-63 Phosphorylation of c-Jun in RAW Macrophages

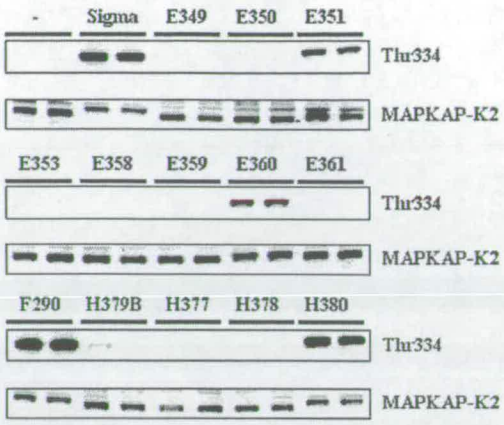


Figure 30 : Effects of Anisomycin and and its Derivatives on the Thr-334 Phosphorylation of MAPKAP-K2 in RAW Macrophages

Compound	R^1	R^2	R^3
E349 (**96**)	Bn	OH	H
E350 (**111**)	Bn	OAc	H
E351 (**112**)	H	OAc	H
E353 (**85**)	H	OH	H
E358 (**88**)	Bn	OH	Me
E359 (**93**)	Bn	OAc	Me
E360 (**94**)	H	OAc	Me
E361 (**83**)	H	OH	Me
F290 (**59**)	H	OCOEt	OH
H379B (**14**)	H	OH	OH
H377 (**56**)	Bn	OH	OBn
H378 (**57**)	Bn	OAc	OBn
H380 (**23**)	H	OAc	OH

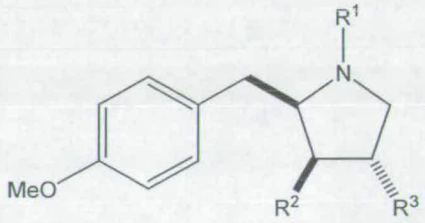


Figure 31 : An SAR Study of Anisomycin on the JNK and p38 Kinase Signalling Pathways

The structure activity relationship study shows that although switching the C(4)-hydroxyl group for a methyl group produces a noticeable decrease in anisomycin's activity, the complete removal of the hydroxyl group has no significant effect (**Figure 31**). These results therefore suggest that the active site of the enzyme is tolerant to changes to the substituent at the C(4) position and are in agreement with previous findings by Jäger.[14]

The study also shows that the presence of an acetate or propionate ester group at the C(3)-*O* position is essential for activity. Removal of this ester reduces anisomycin's activity considerably, and renders the C(4)-Me and C(4)-H analogues **82** and **84** inactive. This lack of activity associated with the lipophilic analogues suggests that deacetylanisomycin's low activity is not a result of its high polarity and implies that deacetylanisomycin is unlikely to be the active form of the drug.

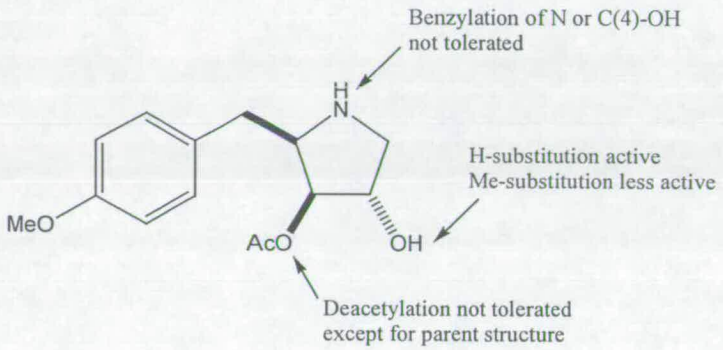


Figure 32 : A Summary of The SAR Study

A comparison of **Figure 32** with **Figure 23** highlights striking similarities between our study and previous SAR studies. In agreement with our study, other studies have also found the removal of the C(3)-*O* ester functionality to be detrimental to anisomycin's biological activity. Similarly, other studies have also found that protection of the secondary amine is not tolerated by the active site of the enzyme, but changes to the substituent at the C(4) position are.[45]

These findings therefore suggest that the active site responsible for the activation of the p38/JNK kinase signalling pathway might be the same as that responsible for protein synthesis inhibition and anti-tumour activity. Shifrin[84] has suggested that anisomycin might activate the JNK pathway *via* a ribotoxic stress response rather than activating the kinase pathway directly.

It is hoped that when our anisomycin analogues are tested for protein synthesis inhibition it will allow us to determine whether this is the case. The study will not only enable us to investigate Shifrin's theory but also allow us to determine whether any of our analogues have the ability to activate the p38/JNK pathway at high concentrations without causing protein synthesis inhibition.

3.3.2 Biological Evaluation of Anisomycin Using a Chemical Genetics Approach

Classical model organism genetics consists of both forward and reverse genetic approaches. In the forward genetic approach the following methods are used to identify genes that regulate a biological process of interest.

- Random mutagenesis of cells or organisms.
- Phenotype-based screening of the cells and organisms.
- Gene identification by mapping of the selected mutations.

In contrast, the reverse genetic approach assigns a biological function to a gene of interest, by employing the following methods:

- The selection of a gene of interest.
- The creation of an organism or cell with mutated version of this gene.
- A broad search for phenotypic differences between the wild-type and mutant organisms or cells.

Although both these approaches are extremely useful for biologists, they have their limitations. Firstly, it is difficult to perform forward analyses in mammalian systems because of the large size of mammalian genomes, their diploid nature and the slow rate of mammalian reproduction. Secondly, genetic mutations, particularly in mammals, cannot easily be turned on and off.

These limitations have led to the development of an alternative approach, known as the chemical genetic approach.[109] In this approach small organic molecules are used, to bind to and alter the function of protein targets. This then either enhances or inhibits the function of the protein.[110]

This approach has the following advantages:

- The mutations produced can readily be turned on or off by simply adding or removing the compounds at will.
- Depending on whether the substrate activates or inhibits the function of their target proteins, they can either act like gain-of-function, or loss-of-function mutations.
- Using this strategy it is possible to screen a vast number of compounds to identify new reagents that act like conditional mutations.
- These small organic molecules can be used to study the mechanistic basis of the phenotype in question, by identifying the protein targets of the reagent.

It was decided to screen our anisomycin analogues against the development of *Xenopus* embryos using the chemical genetic approach.[ξ] This decision was based on the fact that a functional role for activation of the JNK pathway has been determined in dorsal closure, an essential morphogenic process that occurs mid-embryogenesis in *Drosophila*.[111] It was anticipated that the JNK signalling pathway

[ξ] The anisomycin analogues were screened against the development of *Xenopus* embryos in Professor Field's laboratories at the University of East Anglia.

might play a similar role in the growth of *Xenopus* embryos. We were therefore optimistic that our analogues might bind to, and alter the function of a protein target during embryogenesis, to produce some interesting phenotypes.

Preliminary results have found that analogue **56** gives rise to a new and previously unreported phenotype, (**Figure 34**). However, all the other analogues have been found to be either inactive or toxic towards the *Xenopus* embryos at the concentrations used in the initial screens. A comparison of the phenotype with the control shows that the embryos produced have flattened heads and distended bellies.

Interestingly, the SAR study shows that **56** only weakly activates the JNK kinase signalling pathway and does not activate the p38 pathway. In contrast to this, anisomycin has been found to strongly activate both the p38 and JNK pathways but to be highly toxic to the *Xenopus* embryos. This therefore suggests that the selective activation of the JNK pathway might be the cause of this phenotype. A more likely explanation though, is that at the concentrations used in these assays anisomycin, rather than activating the JNK/p38 pathways, instead inhibited protein synthesis in the *Xenopus* embryos causing them to die. It is therefore hoped that a full evaluation of these compounds *via* dose-dependent assays will provide more insight into the mechanisms taking place.

This result suggests that the JNK kinase signalling pathway may contribute to the regulation of multiple biological processes during *Xenopus* embryogenesis. It is hoped that further studies will enable us to identify the protein target of **56**.

Figure 33 : Control Xenopus Embryos

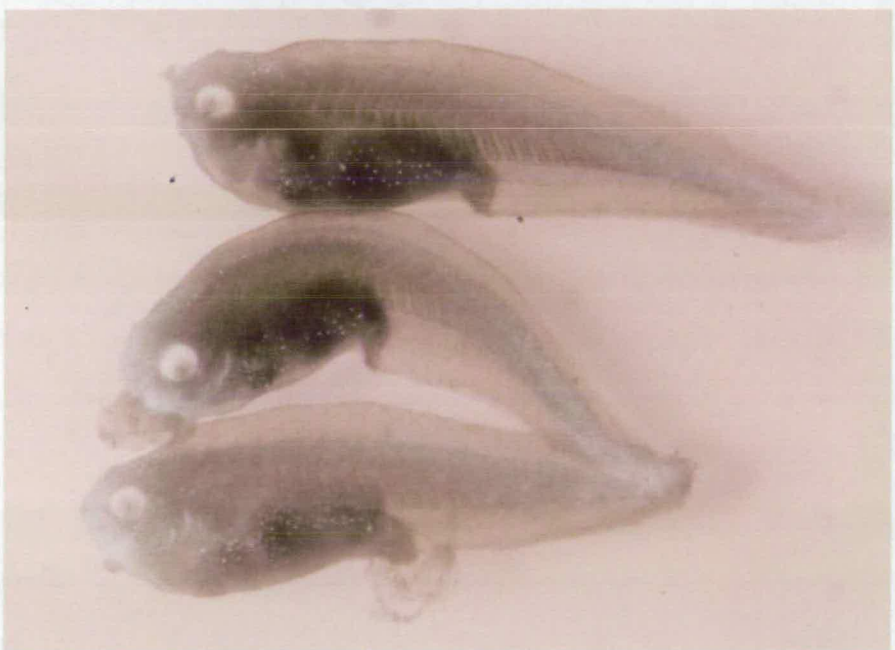


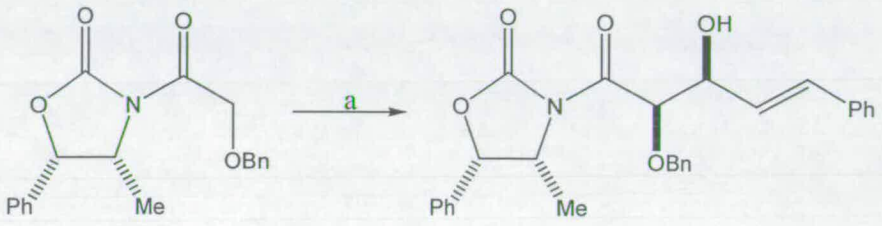
Figure 34 : Phenotype Xenopus Embryos

3.4 Summary of Chapter 3

We have successfully synthesised both the C(4)-Me and C(4)-H derivatives of anisomycin in excellent yield. Assessment of the structure activity relationship of this range of analogues on the p38 and JNK kinase signalling pathways indicated that acylation at C(3)-*O* was vital for activity. It was also found that a number of functional groups may be tolerated at the C(4) position without a significant loss of activity being observed. Finally, using a chemical genetics approach, a synthetic precursor to anisomycin produced a new, previously unreported, phenotype in developing *Xenopus* embryos.

Chapter 4: The Glycolate Aldol Reaction

Optically active 1,2-diol derivatives are useful chiral building blocks, and the development of efficient methods for their preparation is strongly desired. For this reason, interest in the glycolate aldol reaction has increased over the last 15 years. The reaction produces a 1,2-diol by reacting the enolate of an α-hydroxy ketone, amide or ester with an aldehyde, and is often associated with high yields and excellent stereoselectivities.

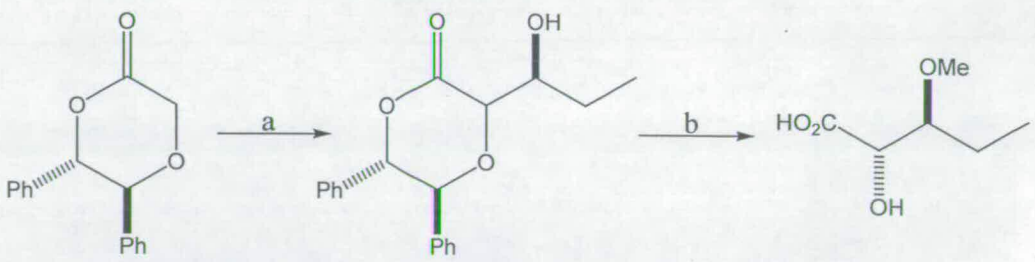


113 **114**

Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) PhCH=CHCHO.

Scheme 41[112(d)]

There are many examples in the literature where *syn* or *anti* aldol products have been obtained from the boron enolate of Evans' oxazolidinone-based glycolate[112,97(b)] (**Scheme 41**). However, problems with *anti* stereoselection often arise because the *E*-enolate geometry required for *anti* aldol formation is not favoured by this or many of the other well known auxiliaries. Consequently in a bid to solve this problem, Andrus recently developed the homochiral diphenyloxapyrone and obtained **117** in 82% yield and with 80% ds[113] (**Scheme 42**).

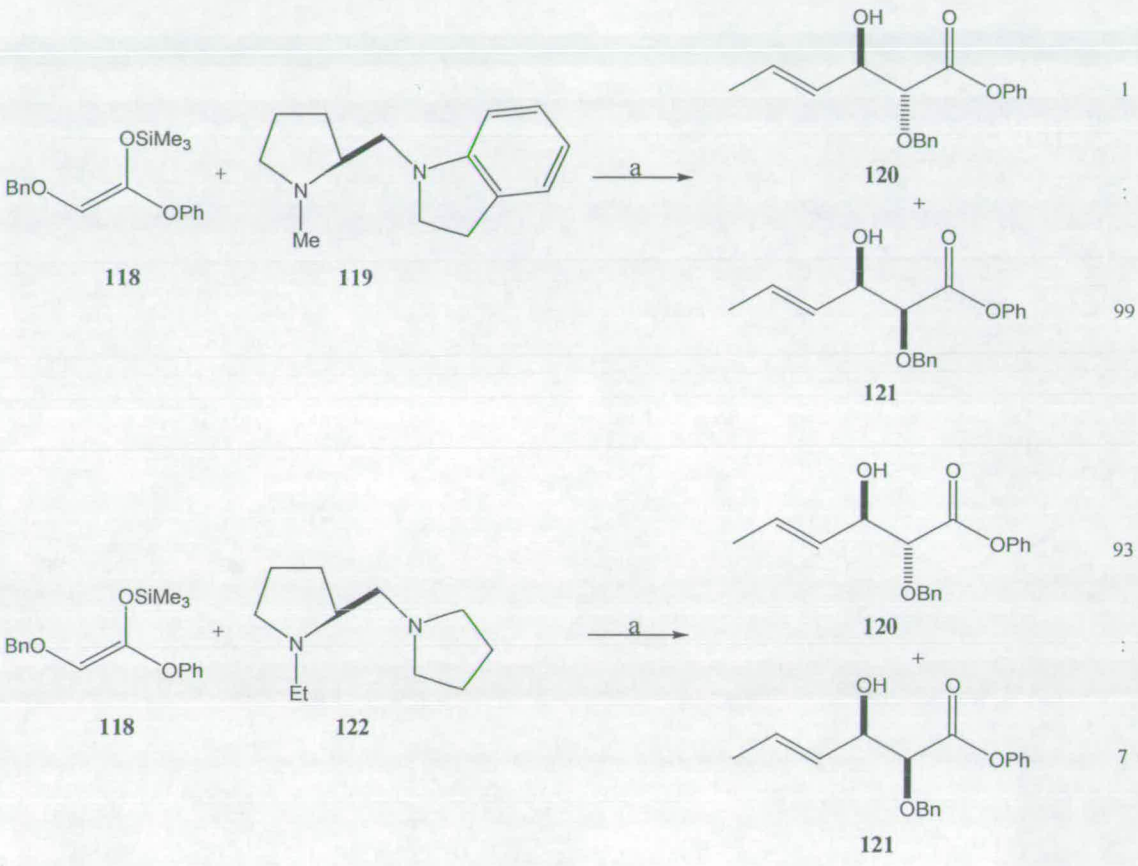


115 **116** **117**

Reagents (a) (i) NEt_3, *c*-Hex_2BOTf, CH_2Cl_2; (ii) Propanal. (b) $Me_3O^+BF_4^-$, H_2, Pd/C.

Scheme 42

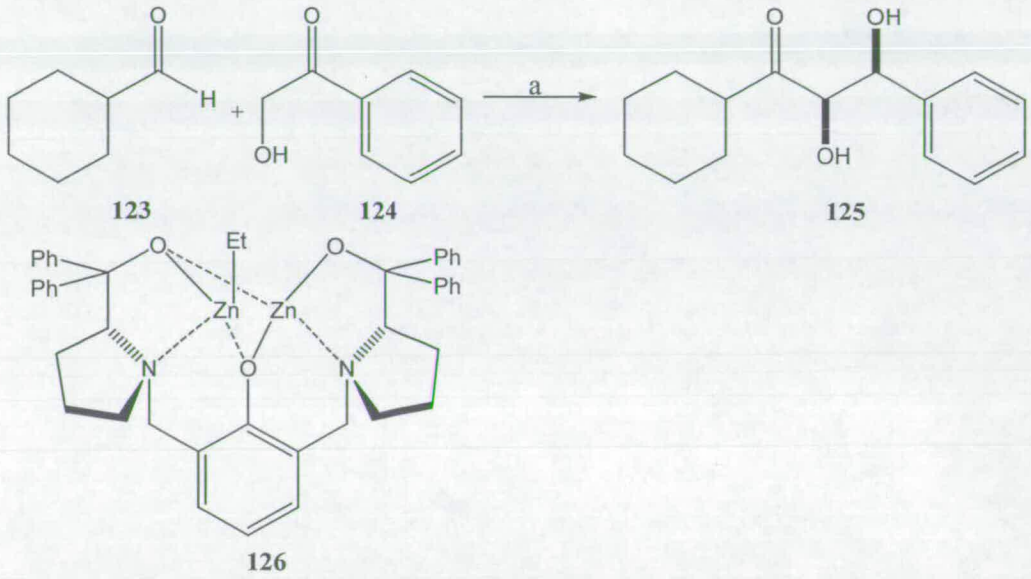
In contrast to the stoichiometric approaches, catalytic glycolate aldol reactions have been very limited with only a few cases having been reported. Perhaps one of the best examples is that of Kobayashi who has used chiral tin Lewis acids to activate the silyl enol ether of α-alkoxy esters to produce either *syn* or *anti* diols with good selectivity (**Scheme 43**).[114]



Reagents: (a) $Sn(OTf)_2$, $Bu_2Sn(OAc)_2$, $CH_3CH{=}CHCHO$, CH_2Cl_2.

Scheme 43

Recently, Trost reported the development of a dinuclear Zinc catalyst **126** (**Scheme 44**).[115] The effectiveness of this catalyst with α-hydroxyketones permitted the use of nearly stoichiometric amounts of both coupling partners in the aldol reaction. Consequently, high yields and excellent stereoselectivities were observed (dr 97 : 3).



Reagents: (a) THF, 4 Å MS.

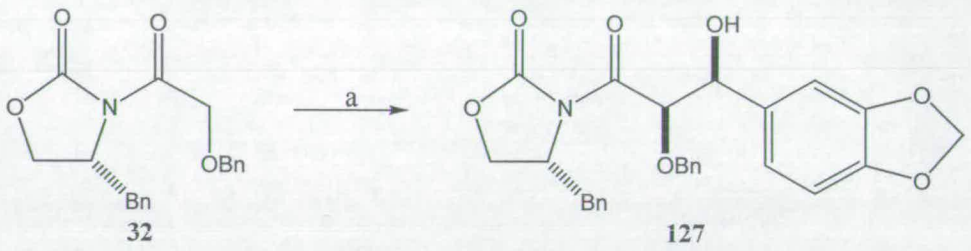
Scheme 44

The osmium-catalyzed asymmetric dihydroxylation reaction provides an alternative approach to 1,2-diol formation. However, unlike the aldol reaction, this approach does not combine the creation of both stereocentres with carbon-carbon bond formation. Similarly, although this approach produces *syn* diols from *E* olefins, its application to *Z* alkenes is severely limited giving predominantly *anti* diols.[116] Therefore, the glycolate aldol reaction has become an invaluable tool for the synthetic organic chemist.

4.1 The Evans' Auxiliary

The Evans' auxiliary is renowned for its use in the aldol reaction. Since its discovery, its use has been linked with high yields and excellent stereoselectivities. Consequently, many research groups have opted to use this auxiliary in an attempt to increase the stereoselectivity of their glycolate aldol reactions.[112]

Kuwano recently employed the glycolate aldol reaction in his synthesis of a key intermediate required for the total synthesis of (2*R*,3*R*)-1,4-Benzodioxane-7-carbaldehyde.[117] He, like others, employed the Evans' auxiliary to impart good levels of stereoselectivity, and obtained aldol adduct **127** in high yield (99%) and with excellent diastereomeric selectivity (>98%) (**Scheme 45**).

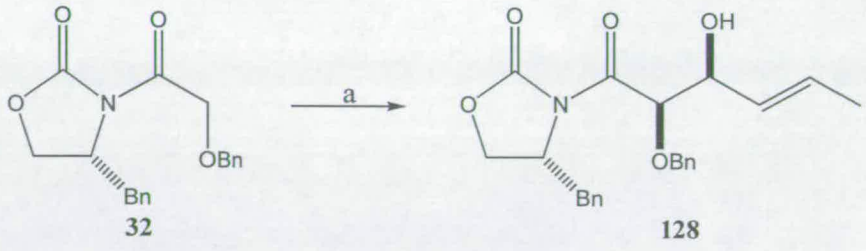


32 **127**

Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) 3,4-methylenedioxybenzaldehyde.

Scheme 45

Holmes also utilised the glycolate aldol reaction during his investigation into the Claisen rearrangement of selenoxides.[97(a)] He too used the Evans' auxiliary to acquire good levels of stereoselectivity, and obtained aldol adduct **128** in 64% yield and with >95% diastereomeric excess (**Scheme 46**).



32 **128**

Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) crotonaldehyde.

Scheme 46

The high stereoselectivities associated with the Evans' auxiliary are the result of a combination of two steric interactions (**Figure 35**). Firstly, the unfavourable interaction of the *E* enolate with the benzyl group on the auxiliary ensures that only the *Z* enolate is formed. This therefore ensures that only the *syn* aldol adduct is obtained when the aldol reaction proceeds *via* a closed 6-membered ring, Zimmerman-Traxler transition state.[118,112(j)]

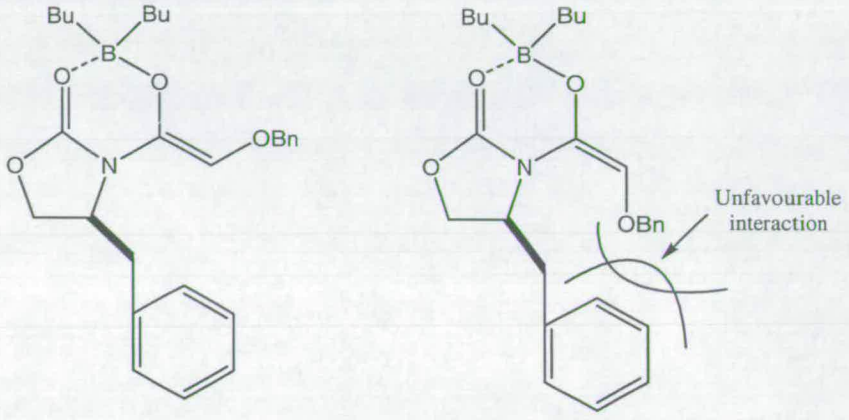


Figure 35 : *Z* vs *E* Enolate Formation Using the Evans' Auxiliary

As well as ensuring the formation of a *syn* aldol adduct, the Evans' auxiliary also determines its absolute stereochemistry. The benzyl group on the auxiliary controls the facial selectivity of the enolate, thereby only allowing an aldehyde to attack at a specific face.

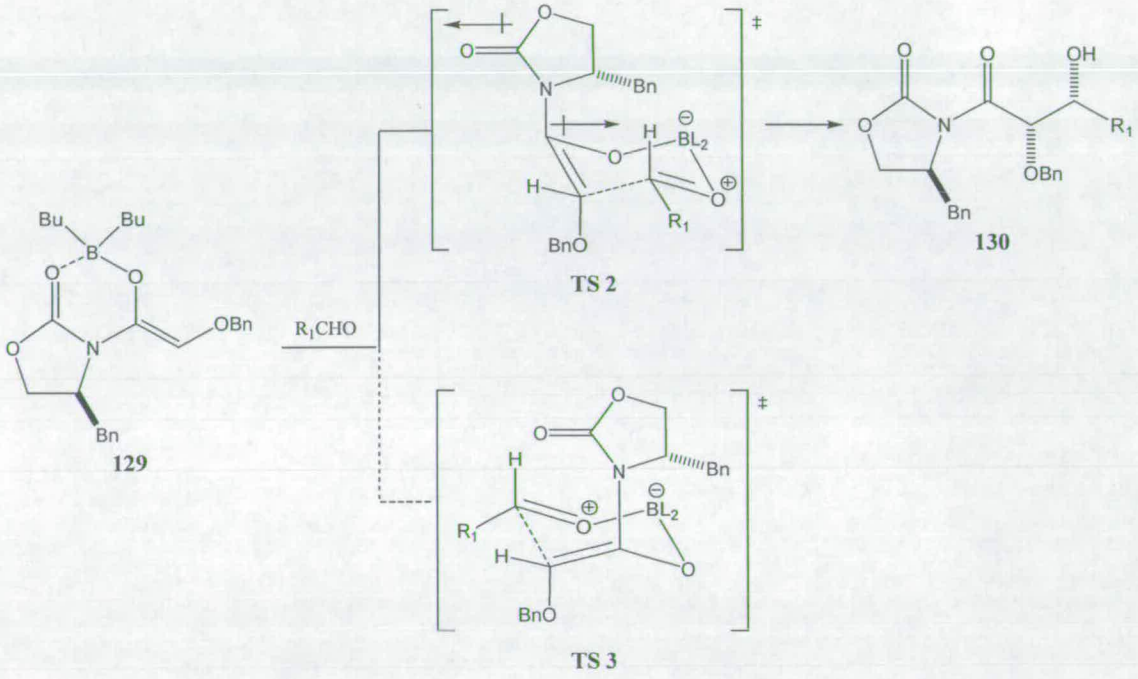


Figure 36 : The Aldol Transition State

The aldol reaction proceeds *via* a Zimmerman-Traxler transition state[119] in which the dipole of the enolate and the auxiliary are opposed (**Figure 36**). When an aldehyde attacks the enolate **129**, only attack on the C_α *si* face is favoured, since an attack on the C_α *re* face forces the benzyl group of the auxiliary over the transition state, and results in unacceptable steric crowding.

4.2 α-Chiral Aldehydes

An alternative approach to increase the level of stereocontrol in the glycolate aldol reaction is to use an α-chiral aldehyde. If a chiral aldehyde can impart good levels of facial selectivity, and control over the enolate geometry can still be maintained, then a useful asymmetric aldol reaction will be produced.

Aldol reactions employing α-chiral aldehydes and achiral enolates again proceed *via* the Zimmerman-Traxler transition state. Here aldehyde **131** can attack the Z-enolate **132** on either face, thereby producing two possible transition states (**Figure 37**).

In the first of the two transition states, significant steric interactions exist between the methyl groups on the aldehyde and the enolate (**TS 4**). Therefore, if the steric requirements of methyl group are smaller than that of the R^1 group, the 'anti Felkin' transition state (**TS 5**) is preferred and results in the formation of aldol adduct **133**.[120]

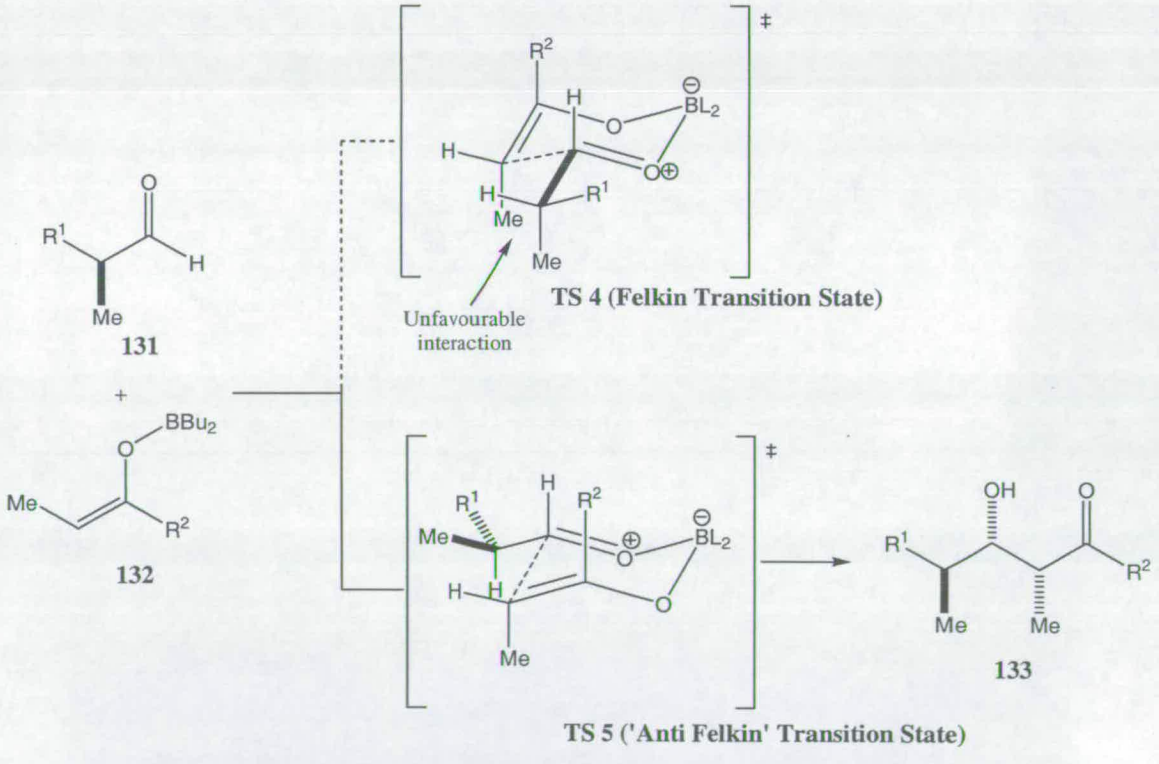
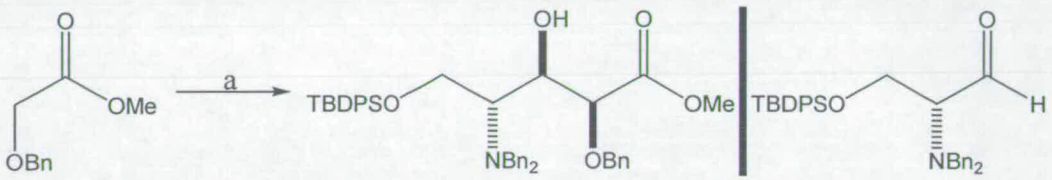


Figure 37 : Transition State Model for (Z)-Enolates with α-Chiral Aldehydes

This suggests that an increase in the steric requirements of R^1 relative to Me will increase the diastereofacial selectivity of the aldol reaction.[120(c)] Consequently, reactions employing *N,N*-dibenzylamino aldehydes derived from amino acids such as glycine should display good levels of facial selectivity. However, by the same rational, the serine derived aldehyde **33**, may suffer from poor facial selectivity due to the small difference in size between its α substituents.

There are surprisingly no examples in the literature of aldol reactions employing achiral glycolate enolates with α-chiral aldehydes. However, during the synthesis of DAB-1, the serine-derived aldehyde **33** was reacted with the achiral methyl ester **134**, to give aldol adduct **135** in high yield (75%) and excellent diastereoselectivity (>98%) (**Scheme 47**).[91]



134 **135** **33**

Reagents: (a) (i) Bu_2BOTf, $^iPr_2NEt$, Et_2O; (ii) **33** (75%).

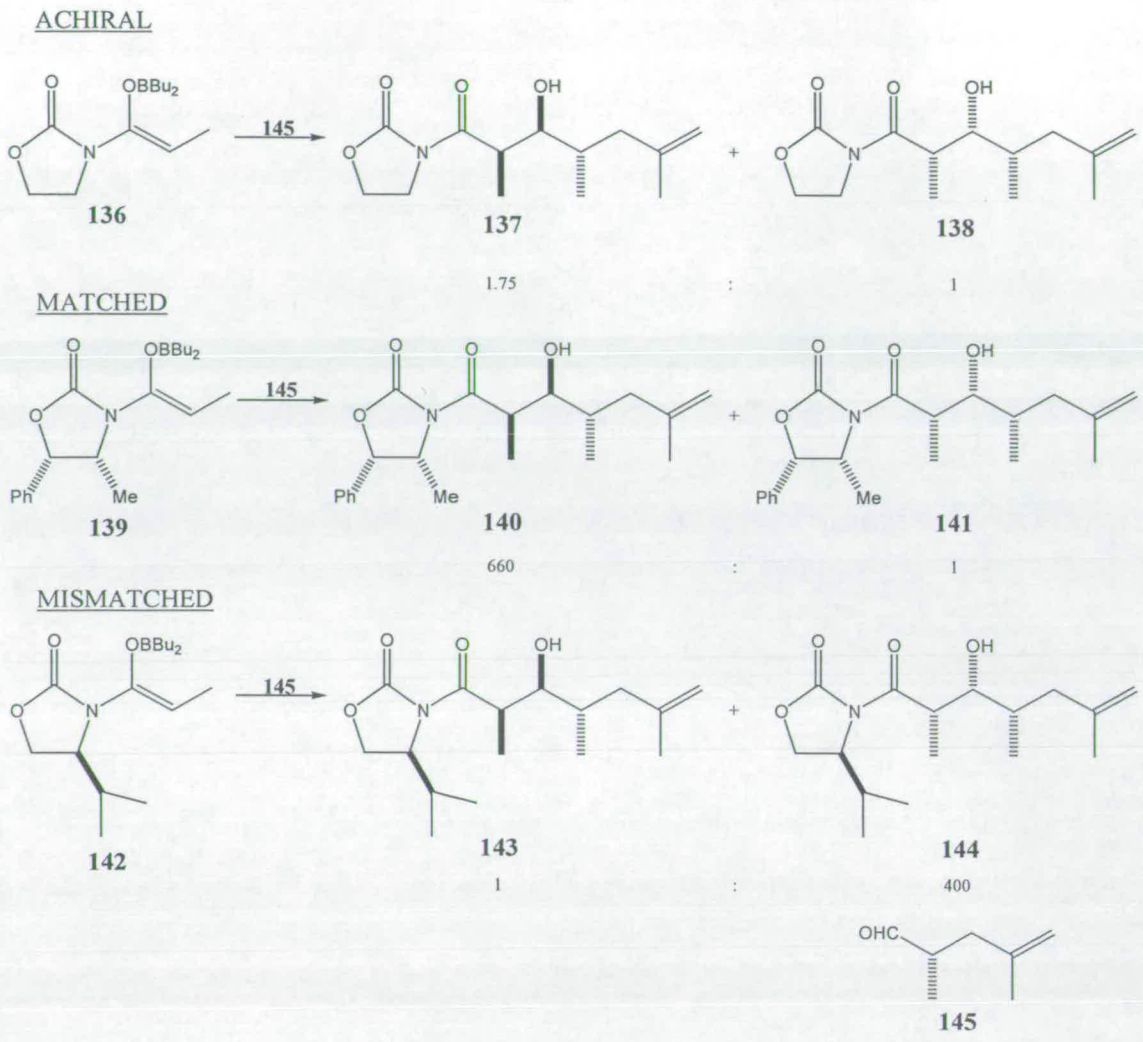
Scheme 47

4.3 Double Asymmetric Induction

When a chiral aldehyde and chiral auxiliary are both employed in an aldol reaction, the two components can either work together to produce the same stereoisomer, or against each other to produce different stereoisomers.

When the two chiral components work together, an aldol adduct is produced with increased stereoselectivity, and the reaction is termed a 'matched' aldol. When the two chiral components compete against each other, it is the auxiliary that usually wins. However, in overturning the stereoinductive capability of the aldehyde, the resulting stereoselectivity observed for the reaction is often less than that for the 'matched' case. Consequently, the reaction is termed a 'mismatched' aldol.

In an investigation into the effects of double asymmetric induction, Masamune subjected aldehyde **145** to a series of 'matched' and 'mismatched' aldol reactions (**Scheme 48**).[121]



Scheme 48

When aldehyde **145** was reacted with achiral enolate **136**, two aldol products **137** and **138** were obtained in a ratio of 1.75 : 1. This low diastereomeric ratio implied that aldehyde **145** only exerted a modest level of stereocontrol in the aldol reaction.

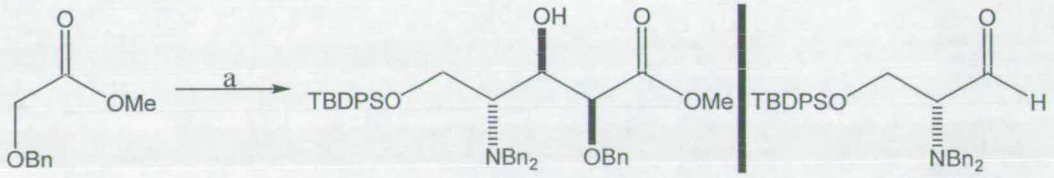
When the same aldehyde was reacted with enolate **139**, the two chiral components worked together to produce the same diastereomer **140** with an increase in diastereoselectivity. However, when the aldehyde was reacted with enolate **142**, the auxiliary completely overturned the stereoinductive capability of **145** to produce the other diastereomer **144** with a diastereomeric ratio of 400:1. Consequently, this reaction was labelled as the 'mismatched' case, and the previous reaction was labelled as the 'matched' aldol.

4.4 Double Asymmetric Induction in the Glycolate Aldol Reaction

The successful investigation by Masamune of double asymmetric induction in the propionate aldol reaction, inspired us to take this a step further and investigate the same concept in the boron mediated glycolate aldol reaction. We wondered if the presence of an additional oxygen atom in the boron enolate would in any way affect the stereoselectivity of the resulting aldol reaction.

4.4.1 Investigation of Double Asymmetric Induction Using the Serine Derived Aldehyde

Previous work within the Hulme group focused on the serine derived aldehyde **33**. Its reaction with the achiral methyl ester **134** displayed high levels of stereoselectivity and gave only a single diastereomer **135** in 75% yield (**Scheme 47**).

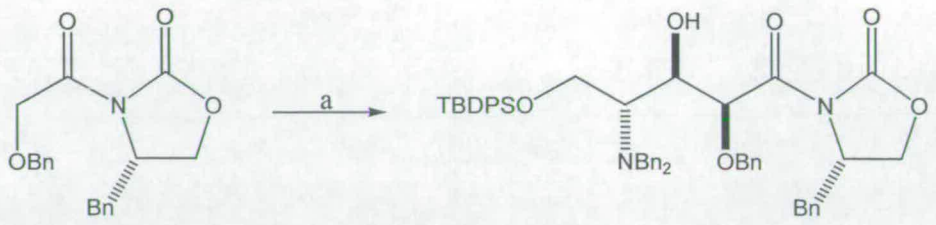


134 **135** **33**

Reagents: (a) (i) Bu_2BOTf, $^iPr_2NEt$, Et_2O; (ii) **33** (75%).

Scheme 47

The reaction of aldehyde **33** with *ent*-**32** also produced a single diastereomer containing the same stereochemistry as **135** (**Scheme 49**). The absence of any other diastereomers suggested that *ent*-**32** and **33** were both working together to produce the same diastereomer **34**. Consequently, the reaction was assigned as the 'matched' aldol.

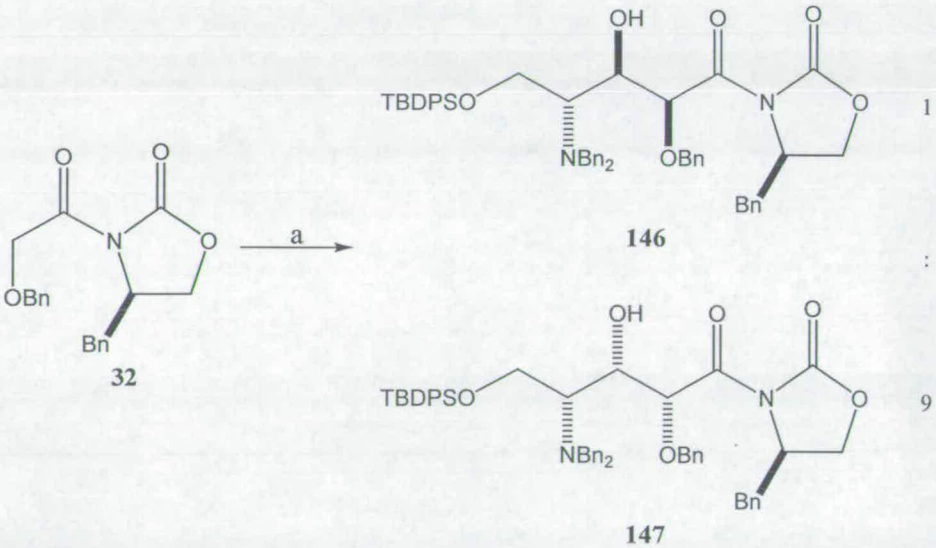


ent-**32** **34**

Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) **33** (82%).

Scheme 49

Due to the high levels of stereoinduction displayed by the serine derived aldehyde, it was anticipated that the remaining aldol reaction might display relatively poor levels of diastereoselectivity. This proved to be the case, with the enolate of **32** failing to completely overturn the stereoinductive capability of aldehyde **33**. Thus, aldol adducts **147** and **146** were produced in a 9:1 ratio with a 79% yield (**Scheme 50**), and the reaction was consequently assigned to be the 'mismatched' aldol.



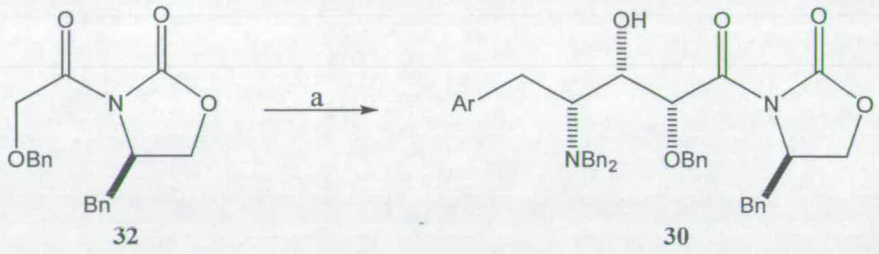
Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) **33** (79%).

Scheme 50

4.4.2 Investigation of Double Asymmetric Induction Using the Tyrosine Derived Aldehyde

With these results in good agreement with those of Masamune, we wished to determine whether other *N,N*-dibenzylamino aldehydes derived from amino acids, displayed the same level of stereocontrol in the glycolate aldol reaction. Consequently, the tyrosine derived aldehyde **31** was subjected to the same set of reactions.

In Chapter 2 we saw that the reaction between aldehyde **31** and glycolate equivalent **32** produced aldol adduct **30** with high stereoselectivity (>95% ds) and good yield (**Scheme 16**). The aldol adduct was then used in the synthesis of anisomycin and this enabled its relative stereochemistry to be confirmed.

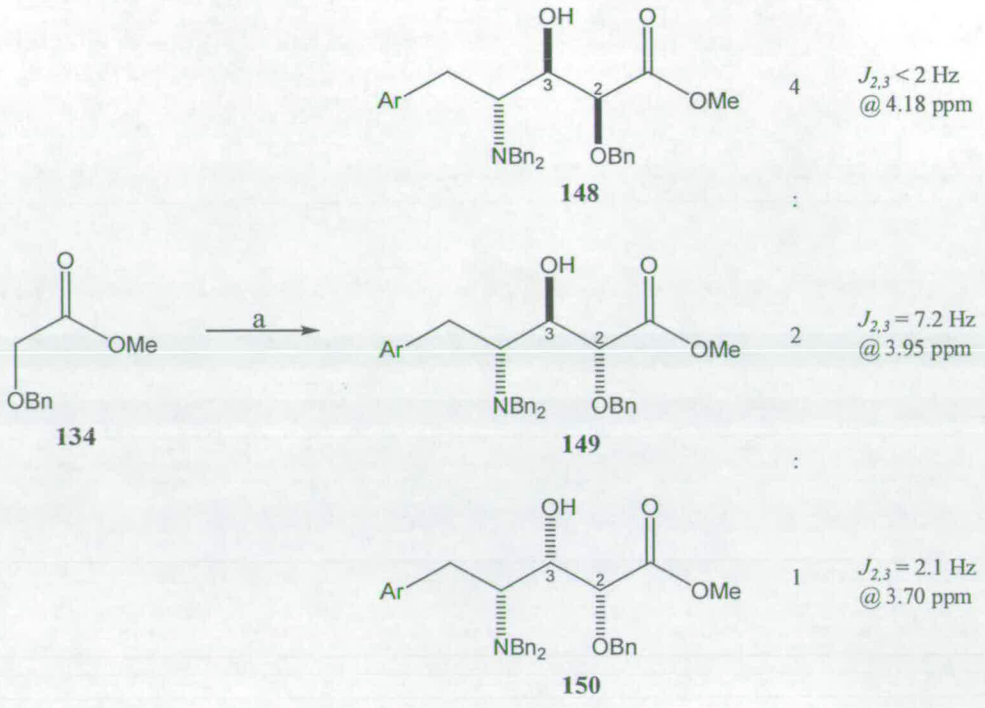


32 **30**

Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) **31**, (75%).

Scheme 16

Although this reaction produced a single product, the analogous reaction using the serine derived aldehyde gave a mixture of two diastereomers (**Scheme 50**). This therefore implied that the stereoinductive capability of the serine derived aldehyde, in working against the directing effects of the Evans' oxazolidione, was greater than that of **31**. It was thus anticipated that the reaction of **31** with the achiral methyl ester **134** would result in relatively low selectivity being observed.

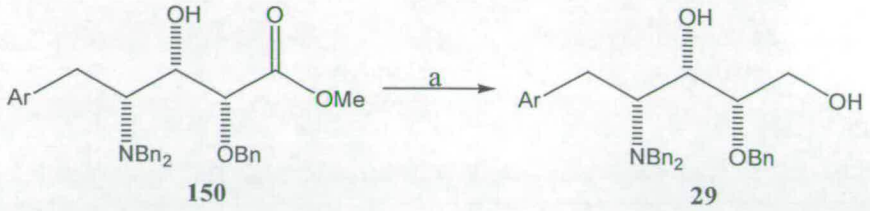


Reagents: (a) (i) Bu_2BOTf, $^iPr_2NEt$, CH_2Cl_2; (ii) **31** (71%).

Scheme 51

The reaction of the achiral methyl ester **134** with aldehyde **31**, gave a mixture of three aldol adducts which were tentatively assigned the structures shown (**Scheme 51**). The fact that three aldol adducts had been obtained, indicated that at least one of them had to be an *anti* aldol product. The presence of a characteristic *anti* aldol peak (δ = 3.95 (d, $J_{2,3}$ = 7.2 Hz)) in the ^1H NMR of **149** suggested that this compound was the likely candidate. In a bid to determine the structure of all three aldol adducts, several chemical transformations were carried out.

The minor diastereomer **150** was reduced with lithium aluminium hydride, to give a diol which was identical in all respects (NMR, IR, $[\alpha]_D$, R_f) to diol **29**, produced during the synthesis of anisomycin (**Scheme 52**). It was therefore concluded that the minor diastereomer had been assigned the correct structure.

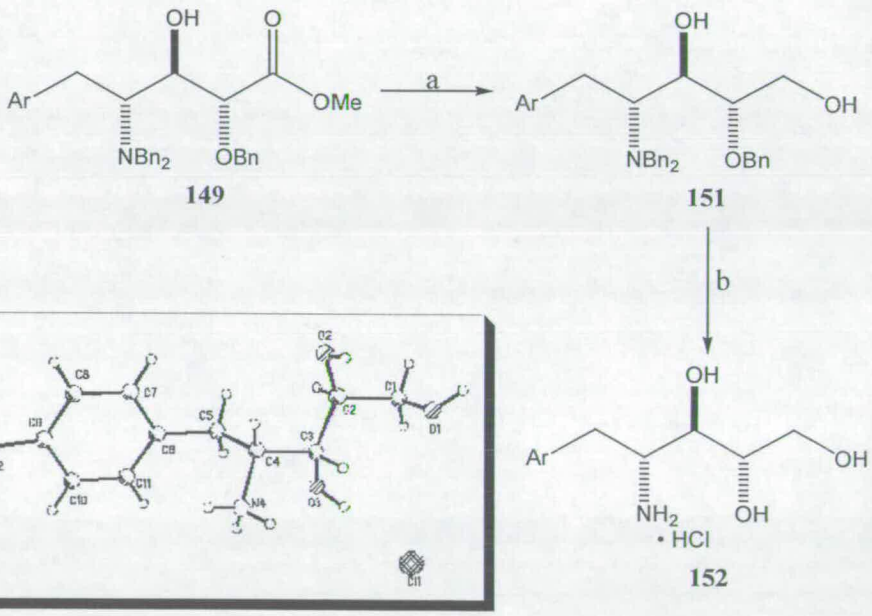


150 29

Reagents: (a) $LiAlH_4$, THF (72%).

Scheme 52

The aldol product suspected of having *anti* stereochemistry, was also reduced using lithium aluminium hydride. The resulting diol **151** was then fully deprotected in the presence of 1 M hydrochloric acid to give the amine as its hydrochloride salt (**Scheme 53**). An X-ray crystal structure of the salt confirmed that **152** was indeed derived from the *anti* aldol adduct **149**, and had thus been assigned the correct stereochemistry.



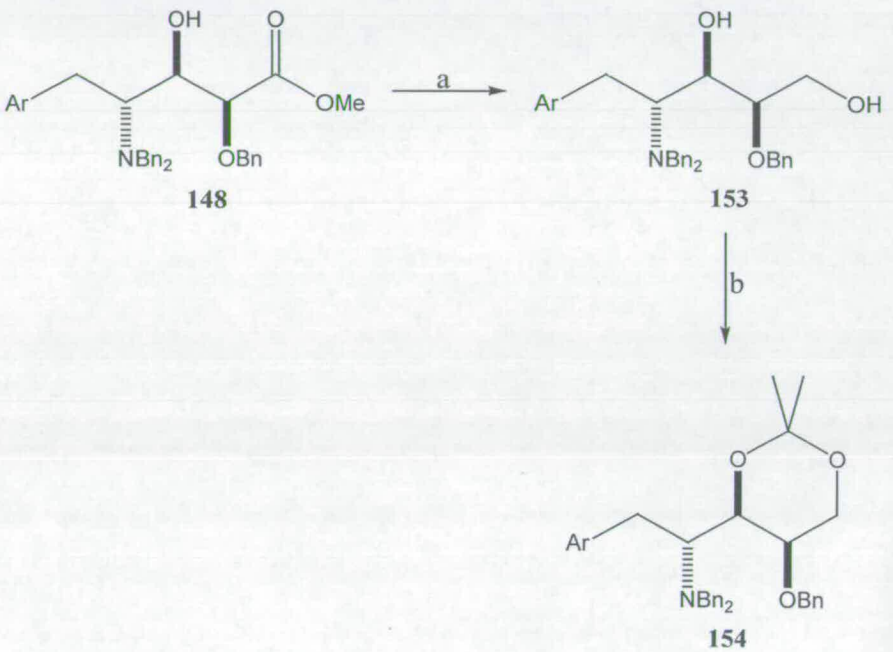
149 151 152

Reagents: (a) $LiAlH_4$, THF (78%); (b) H_2, 20% $Pd(OH)_2/C$, 1 M HCl/Et_2O (100%).

Scheme 53

Having determined **148** to be a *syn* aldol adduct using ^{1}H NMR (δ = 4.18 (s, $J_{2,3}$ < 1 Hz), and having correctly assigned the other *syn* aldol adduct, we were confident that the major diastereomer had been assigned the correct structure. However, as a final precaution, we set about confirming its relative stereochemistry.

Initial efforts focused on a similar protocol to that employed for the *anti* diastereomer. However, deprotection of diol **153** failed to give a crystalline salt. Consequently, our attentions turned towards converting the diol to its acetonide. This was achieved, with limited success, by treating diol **153** with acetone in the presence of iodine (**Scheme 54**).[122]



Reagents: (a) $LiAlH_4$, THF (67%); (b) $(CH_3)_2CO$, I_2 (30%).

Scheme 54

Although a range of conditions are appropriate for converting a diol to its acetonide,[123] our choice of reagents was based upon previous success within the Hulme group. However, these conditions failed to produce the acetonide in high yield, and unfortunately, due to time constraints, we were unable to repeat this experiment using different conditions.

The small coupling constants observed in the ^{1}H NMR of **154**, suggested that the interactions of H* with its adjacent protons were all either axial-equatorial or equatorial-equatorial. Using this information, the acetonide was assigned the structure shown in **Figure 38** and this therefore again confirmed that the assigned structure of **148** was in fact correct.

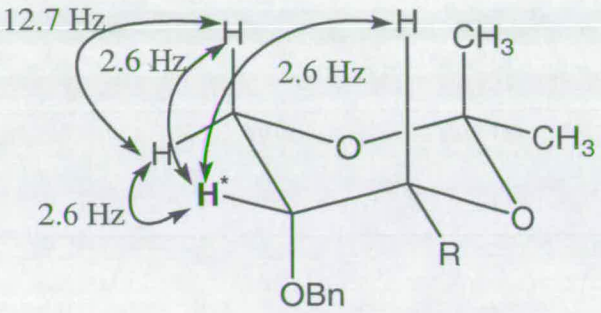
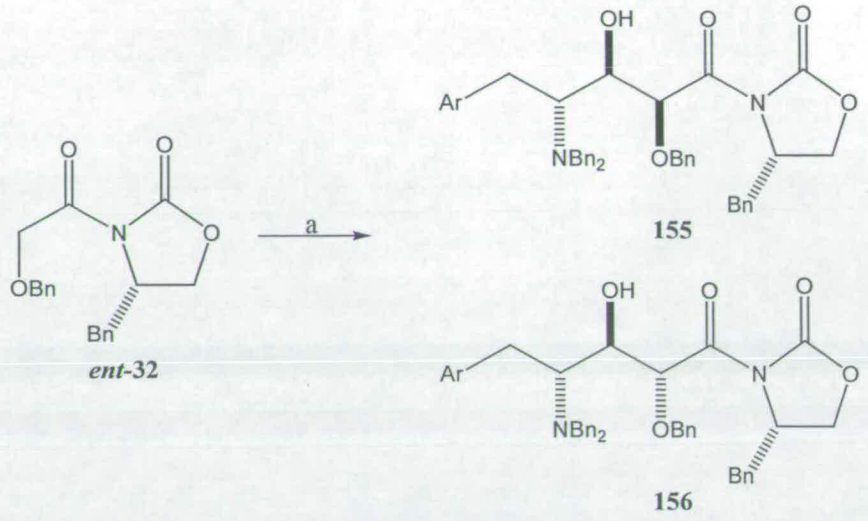


Figure 38 : ^{1}H NMR Coupling Between Adjacent Protons in 154

With the structure of all three aldol adducts having been confirmed, it was clear that the achiral glycolate aldol reaction had not only produced the two *syn* aldol adducts as anticipated, but it had also produced a significant amount of an *anti* aldol product.

The two *syn* aldol products were produced in a 4:1 ratio, which was in marked contrast to the analogous reaction using the serine derived aldehyde, where only a single diastereomer was observed. This therefore allowed us to conclude that stereoinductive capability of aldehyde **31** was significantly less than that of its serine counterpart.

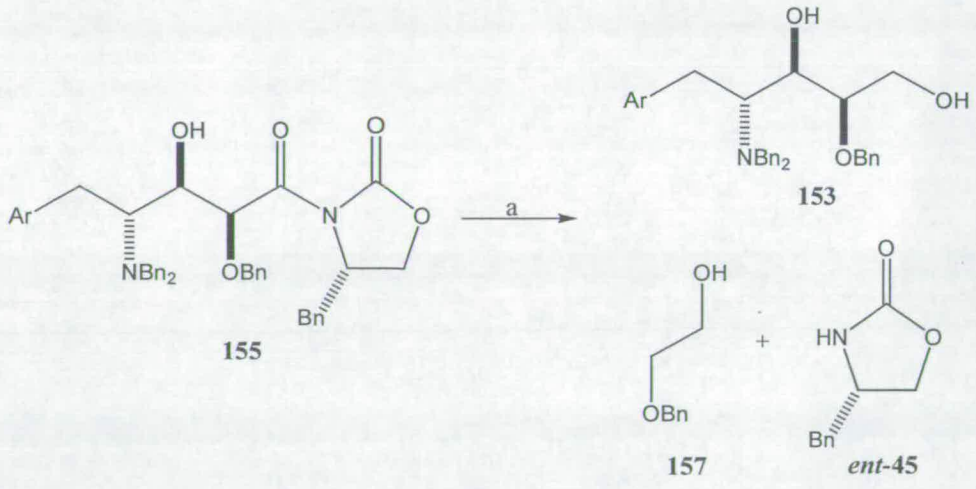
In order to complete our set of experiments, aldehyde **31** was reacted with imide *ent*-**32** to give two products, which were tentatively assigned the structures shown (**Scheme 55**).



Reagents: (a) (i) Bu_2BOTf, NEt_3, CH_2Cl_2; (ii) **31**.

Scheme 55

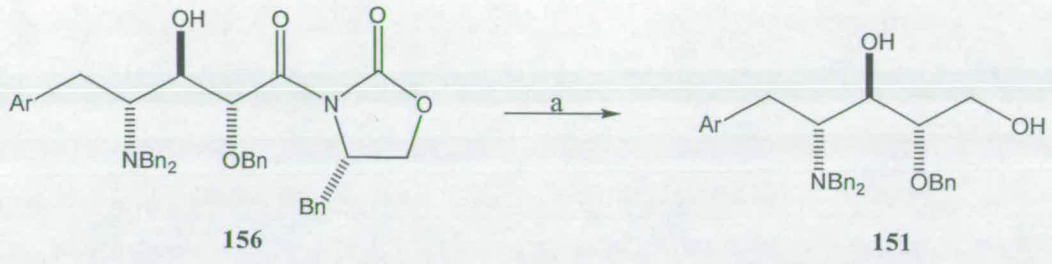
Despite various efforts using different chromatographic techniques, the major diastereomer **155** could not be isolated from recovered unreacted acylated auxiliary *ent*-**32**. Therefore, in an attempt to determine the relative stereochemistry of **155**, the mixture was reduced, and a diol was isolated cleanly. The diol obtained was identical in all respects (NMR, IR, $[\alpha]_D$, R_f) to diol **153**, and was therefore confirmed to have the tentatively assigned structure (**Scheme 56**).



Reagents: (a) $LiBH_4$, MeOH, THF.

Scheme 56

The minor diastereomer was reduced to give a diol using the same procedure as above. Again, full analysis (NMR, IR, $[\alpha]_D$, R_f) revealed that the diol had the same structure as diol **151** and had therefore been derived from the *anti* aldol adduct **156** (**Scheme 57**).



156 **151**

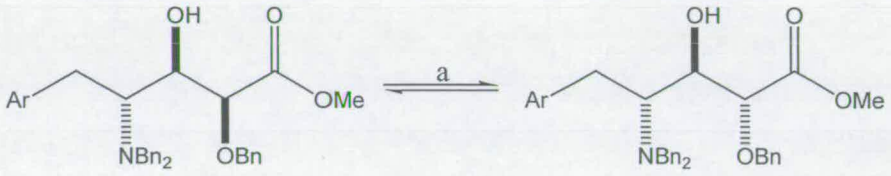
Reagents: (a) $LiBH_4$, MeOH, THF (75%).

Scheme 57

The fact that this aldol reaction had proceeded to give a single *syn* diastereomer, meant that it was in good agreement with the analogous reaction using the serine derived aldehyde **33**. Therefore, with all three aldol reactions having been successfully carried out, it was concluded that the aldol reaction used in the synthesis of anisomycin was in fact the 'mismatched' case, and the aldol reaction described above was therefore the 'matched' case.

4.5 Investigation Into *anti* Aldol Product Formation

The generation of a single *anti* diastereomer in both the 'matched' and achiral aldol reactions was thought unlikely to be a consequence of *E* enolate formation for reasons discussed earlier. As an alternative explanation for this *anti* aldol formation, it was envisaged that aldol adducts **148** and **155** may be undergoing epimerisation following their formation (**Scheme 58**).



148 **149**

Reagents: (a) Bu_2BOTf, NEt_3, CH_2Cl_2.

Scheme 58

To ascertain whether this was the case, aldol adduct **148** was re-subjected to the reaction conditions. However, after the usual work up, ^1H NMR revealed that no appreciable epimerisation had occurred, and this therefore suggested that *anti* aldol formation was occurring *via* a different mechanism.

It was thought possible that the *anti* aldol adduct might have been formed *via* an open transition state.[124,118(e)] When excess dibutylboron triflate is present in the aldol reaction it can itself form an activated aldehyde complex. This prevents the formation of the Zimmerman-Traxler transition state and instead produces an open transition state, in which the enolate boron is chelated to the oxazolidinone carbonyl.

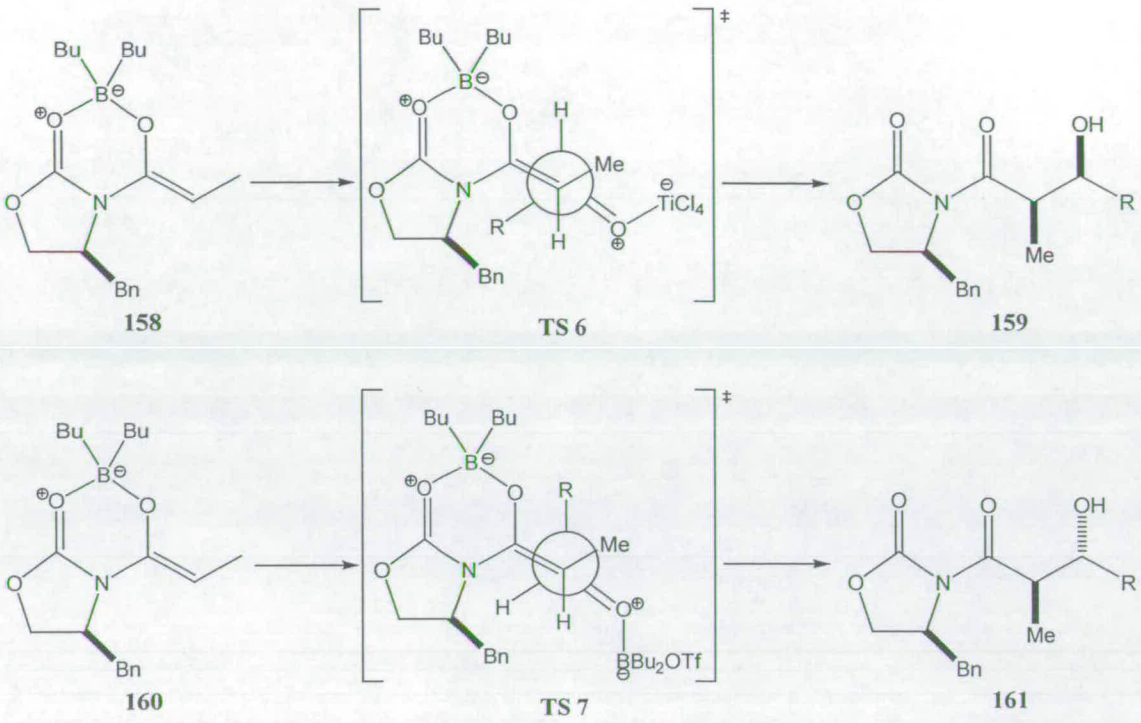


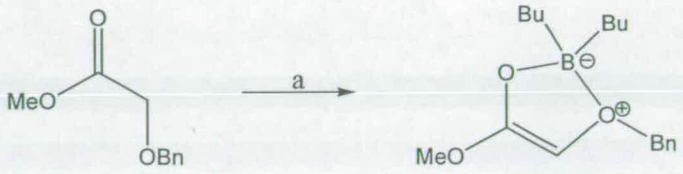
Figure 39 : The Aldol Open Transition State

Although the facial selectivity of the auxiliary in the open transition state is the same as that observed in alkylation reactions, the stereoisomer produced also depends on the size of the Lewis acid employed (**Figure 39**).

If the Lewis acid is relatively small, the aldol reaction proceeds *via* **TS 6** and results in the formation of the 'non-Evans' *syn* aldol adduct **159**. On switching to a larger Lewis acid, steric interactions between the acid and the enolate increase, forcing the reaction to proceed *via* **TS 7**. This results in the formation of the *anti* aldol adduct **161** and could therefore account for the formation of aldol adduct **156** in our glycolate aldol reactions.

Reports in the literature suggest that the auxiliary's presence is important for *anti* aldol formation to occur *via* the open transition state.[124a] However contrary to this, we found that *anti* aldol formation occurred irrespective of the auxiliary's presence.

One possible explanation is that the dibutylboron triflate chelates to both the oxygen atoms in the methyl ester **134** to produce enolate **162** (**Scheme 59**). This internal chelation then allows an activated aldehyde complex to attack the enolate in a similar manner to that shown in **Figure 39**.



134 **162**

Reagents: (a) Bu_2BOTf, $^iPr_2NEt$, CH_2Cl_2.

Scheme 59

Although this explanation accounts for the formation of the *anti* aldol adduct **149**, it fails to explain why only a *syn* aldol product was obtained when the serine derived aldehyde **33** was employed in an analogous reaction.

This may be explained however, by considering the choice of reaction solvent. With a Lewis basic solvent, e.g. ether, a strong complex is formed with the excess dibutylboron triflate which may prevent it from catalysing the formation of *anti* aldol products.[124a]

This suggests that in the aldol reaction employing the serine derived aldehyde **33**, the use of ether as the solvent is likely to have prevented the formation of *anti* aldol products. Unfortunately, the aldol reaction employing the tyrosine derived aldehyde **31** was conducted in CH_2Cl_2 due to the aldehyde's insolubility in ether. It is possible therefore, that this switch in solvent from ether to CH_2Cl_2 may have been responsible for the formation of the *anti* aldol product.

Having conceded that the use of CH_2Cl_2 was the most likely reason for the formation of *anti* aldol products, efforts began focusing on trying to force the aldol reaction to go *via* the closed transition state. It had been proposed that the use of triethylamine, being less hindered, would form a stronger complex with excess dibutylboron triflate than with diisopropylethylamine.[124a] It was hoped that the

formation of this complex would therefore prevent the Lewis acid from catalysing the *anti* aldol reaction. However, efforts to remove excess dibutylboron triflate by adding triethylamine failed to force the reaction to go *via* the closed transition state. Similarly, attempts to see if varying the dibutylboron triflate concentration in the reaction mixture would have any effect on the *syn*:*anti* ratio also failed.

It therefore seems likely, with epimerisation ruled out as a possible explanation, that the open transition state might be responsible for the formation of the *anti* aldol products. However, many questions still remain unanswered. For example, why did the 'matched' aldol reaction produce *syn* products with one aldehyde, but give a mixture of *syn/anti* products with another? Similarly, why did the addition of excess triethylamine or dibutylboron triflate not have any noticeable effect on the products of the achiral reaction? Consequently, in a bid to resolve these issues, ongoing work is continuing within the Hulme group in the hope of finding a suitable explanation for these problems.

4.6 Summary Of Chapter 4

The tyrosine derived aldehyde shows that there is a fine balance between different elements of stereocontrol in the glycolate aldol reaction of *N,N*-dibenzylamino aldehydes. Some of the possible explanations for the *anti* aldol adduct observed in this reaction have been investigated.

Chapter 5 : The Synthesis of a 1-Deoxynojirimycin Analogue

The Hulme group has successfully employed both the 'matched' and 'mismatched' glycolate aldol reactions in the synthesis of DAB-1 (**11**), nectrisine (**163**) and anisomycin (**10**). We have also exploited the aldol reaction in order to access the C(4)-Me derivatives and by substituting the Claisen condensation for this reaction, we have been able to access the C(4)-H derivatives (**Figure 40**). Our ability to vary these parameters has not only enabled us to synthesise three natural products, but it has also allowed us to produce a series of analogues.

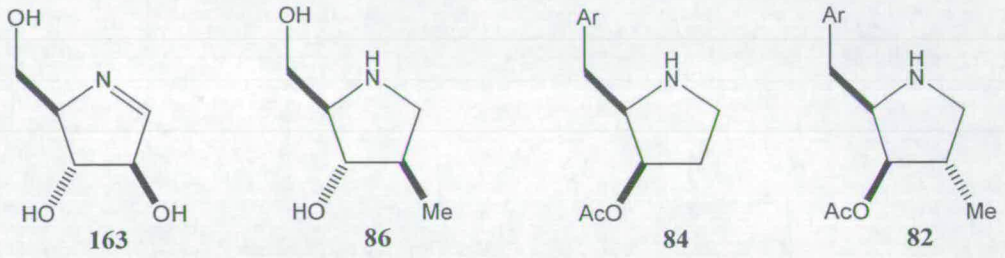
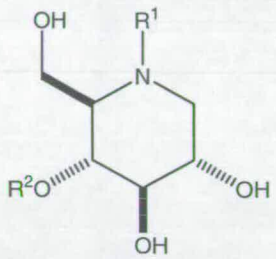


Figure 40 : Iminosugars Synthesised Within the Hulme Group

Having obtained this versatile and robust route to the five membered iminosugars, we were interested in extending our methodology to include the synthesis of their six membered counterparts. We hoped that a similar approach would enable us to synthesise the azasugars in good yield and also allow us to access some of their analogues.

5.1 1-Deoxynojirimycin

One azasugar, which particularly caught our attention, was 1-deoxynojirimycin (DNJ) **164**. DNJ was isolated from *Streptomyces nojiriensis* in 1967[125] and has since shown potential as a chemotherapeutic agent for treating diabetes, cancer and viral infections (**Figure 41**).[126] Of similar interest to us, was the fact that its derivatives have been shown to inhibit the development of HIV *in vitro*.[127] It was hoped that if we could produce a synthetic route to the sugar, that had the same propensity for producing analogues as our previous syntheses, then this would enable us to produce a library of novel analogues with interesting biological properties.



Compound	Activity	Biological organism	IC_{50} (μg/ml)	R^1	R^2
164	HIV[128]	Moloney murine lukemia	1.2-2.5	H	H
164	Glucosidase II[129]	Mung bean microsomes	2-5	H	H
164	Sucrase[130]	Beagle dog small intestine	0.12	H	H
165	Cyclodextrin glycosyltransferase[131]	*Bacillus stearothermophilus*	>200	Bn	H
166	Maltase[130]	Rabbit small intestine	0.46	CH_3	H
167	Maltase[132]	Rabbit small intestine	170	CH_3	α-D-glu

Figure 41 : 1-Deoxynojirimycin and its Biological Properties

5.2 Previous Syntheses of 1-Deoxynojirimycin

With the discovery over the last 20 years that DNJ possesses various different biological properties, synthetic interest in the natural product has increased. Over 15 syntheses of the natural product have been reported. Many of these syntheses rely on the chiral pool, however a few asymmetric syntheses have also been reported (**Figure 42**).[133]

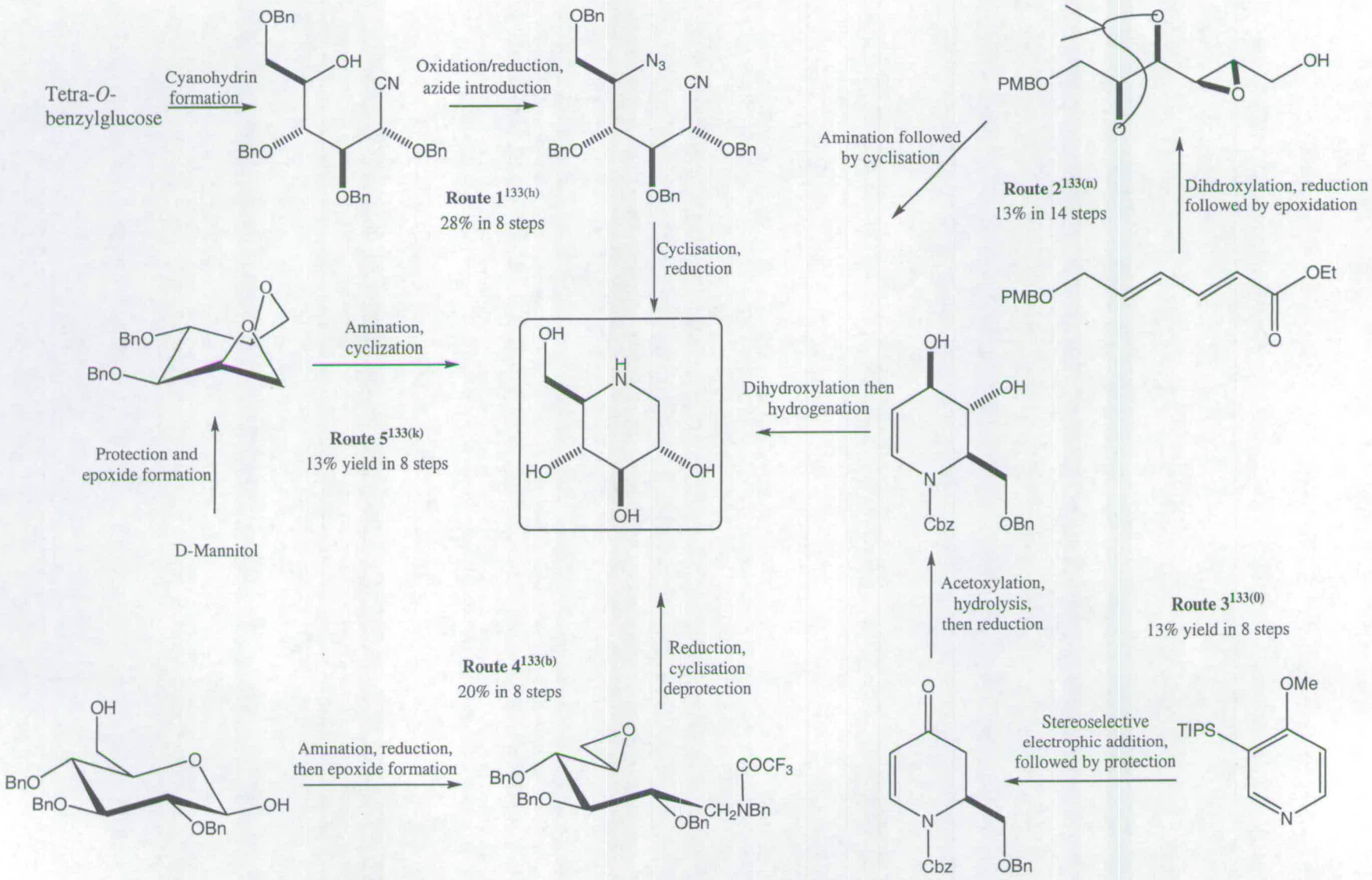
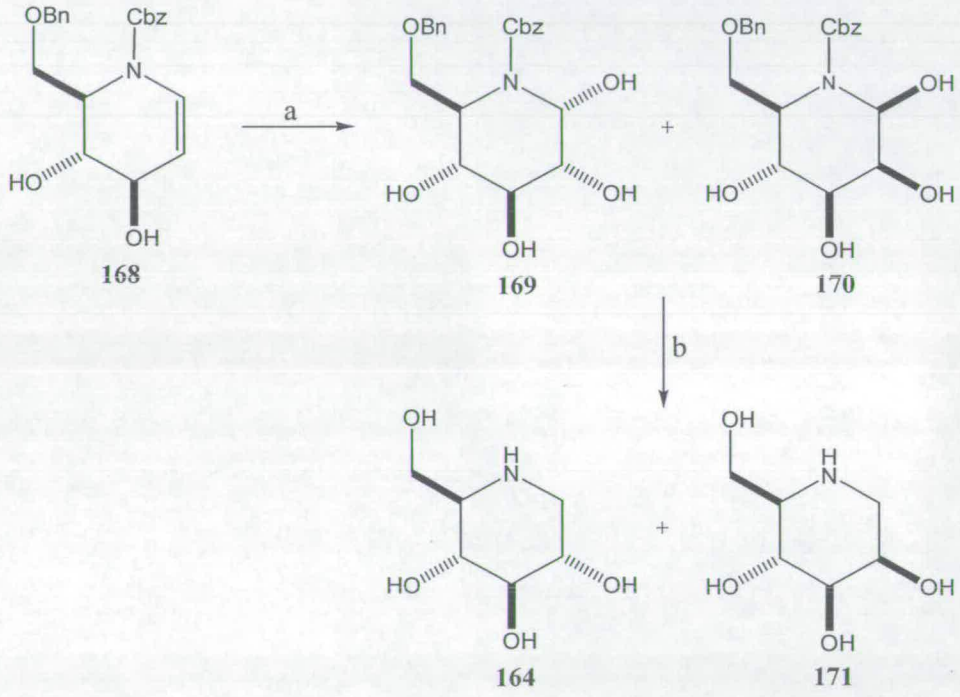


Figure 42 : Previous Syntheses of DNJ

Syntheses of DNJ are generally short and direct, and often require less than 10 synthetic steps. However, as a consequence, the efficiency of these routes is often poor and overall yields less than 15% are frequently reported.

One reason for these low yields is due to the employment of poor stereoselective reactions. This is particularly highlighted in route 3 where the asymmetric dihydroxylation reaction gives the diols **169** and **170** in a 3:1 ratio (**Scheme 60**). These two diastereomers obtained were then found to be unstable to silica gel and therefore were subjected to hydrogenation conditions crude. This resulted in a difficult chromatographic purification (MeOH:CH_2Cl_2:TEA, 75:24:1) and consequently, DNJ was only isolated in 55% yield over these two steps.

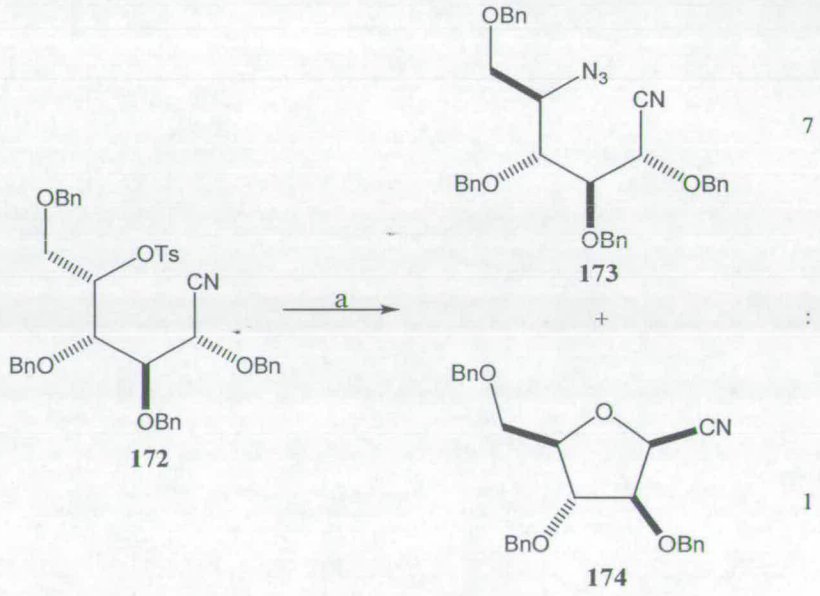


Reagents: (a) OsO_4, NMO; (b) $Pd(OH)_2/H_2$, 10% HCl (aq.).

Scheme 60

Vasella and co-workers chose to synthesise DNJ from glucose (route 1). However in doing so, they had to ensure that they maintained the glucose stereochemistry at C(1). Consequently, prior to the S_N2 attack by sodium azide, the stereochemistry at C(1) had to be inverted. This was achieved using an oxidation/reduction protocol, but this therefore added a further two steps onto their synthesis.

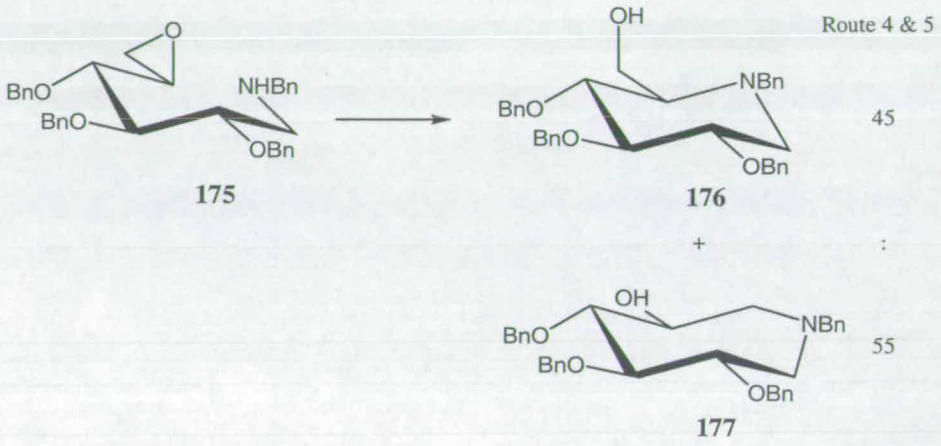
The displacement of the tosylate group using sodium azide also proved problematic. The reaction not only gave the azide **173** but also produced 2,5-anhydro-D-gluconononitrile **174** in 10% yield (**Scheme 61**). Therefore, the formation of this side product in significant yield again compromised the efficiency of the synthesis.



Reagents: (a) NaN_3, DMSO.

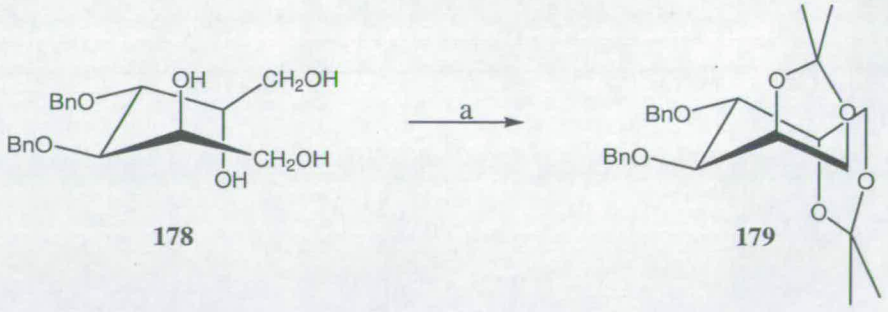
Scheme 61

DNJ has also been synthesised in 8 steps from D-glucose and D-mannitol. In both these syntheses the piperidine ring was formed by an amine attacking an epoxide in order to produce the primary alcohol at the 1-position. However, this cyclisation also gave a seven membered ring in considerable yield, and this therefore resulted in a poor yield of DNJ being isolated in both cases (**Scheme 62**).



Scheme 62

Both these syntheses rely heavily on the built-in chirality and functionality of their starting materials. This therefore severely limits their potential for producing a wide range of analogues for biological testing. Similarly, biological systems are extremely sensitive towards tin. Therefore the use of tin chloride in route 5 to selectively protect the 1,2 and 5,6-diols in D-mannitol will cast into doubt the validity of biological results obtained using DNJ, or any of its analogues synthesised by this route (**Scheme 63**).



Reagents: (a) $Me_2C(OMe)_2$, $SnCl_2$, $(CH_2OMe)_2$.

Scheme 63

In conclusion, previous syntheses of DNJ have tended to be low yielding and have invariably suffered from poor stereoselective reactions. Their focus for being short and direct has led to many of these syntheses being rigid, and therefore incapable of offering the flexibility required to produce a large range of analogues. Therefore, bearing all of this in mind, we set about devising our own retrosynthetic analysis.

5.3 Retrosynthesis of 1-Deoxynojirimycin

Our retrosynthetic analysis of DNJ is shown in **Figure 43**. The retrosynthesis is based around aldol adduct **34,** whose synthesis within the Hulme group has already been described. (*cf.* Chapter 4)

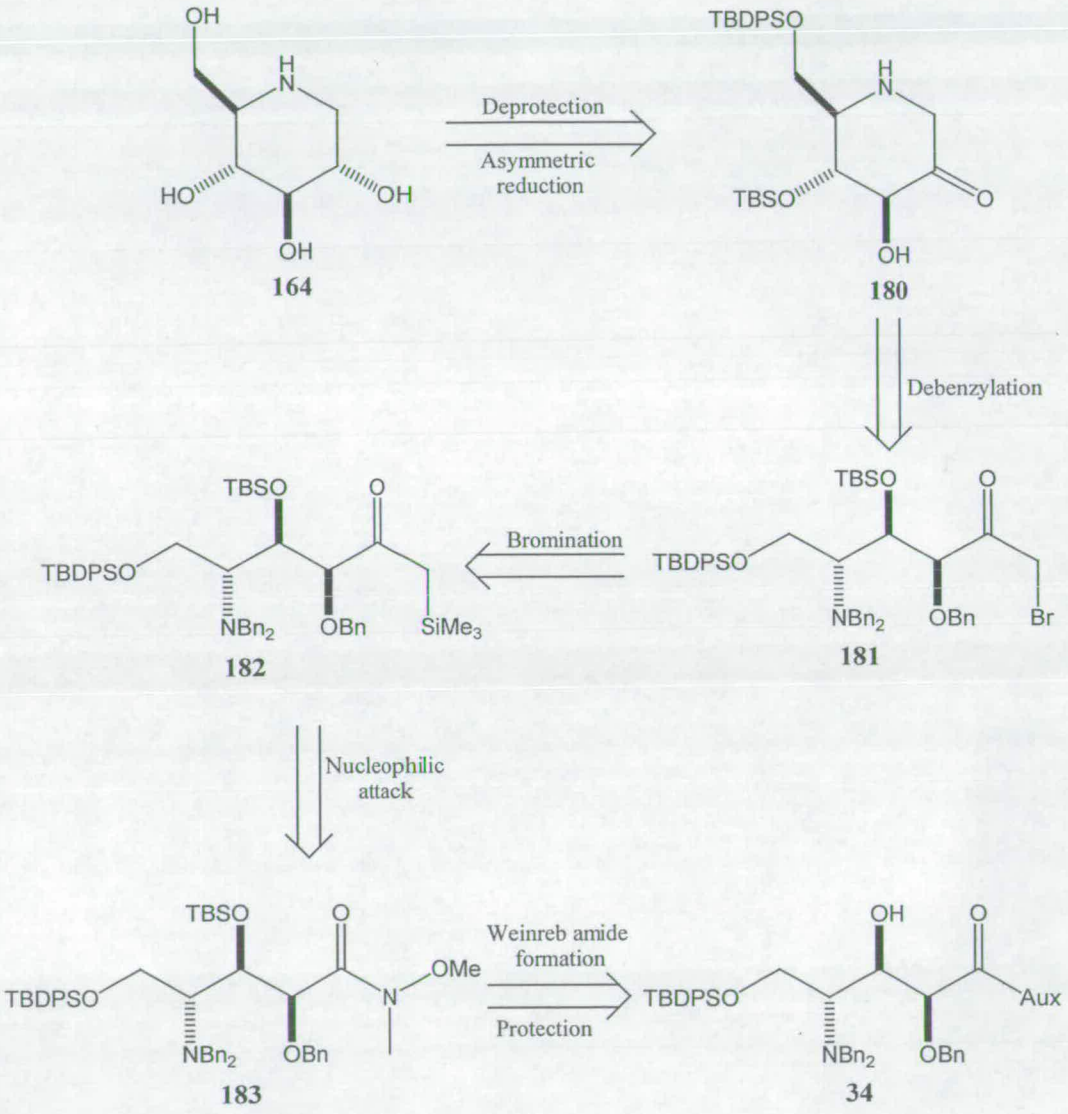
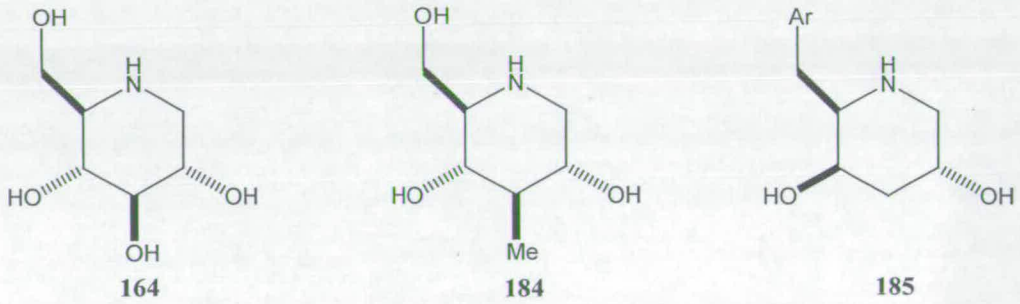


Figure 43 : The Retrosynthesis of 1-Deoxynojirimycin.

Previous work within the group suggested that the Weinreb amide formation from **34** and subsequent protection of the secondary alcohol would proceed smoothly to give **183**. From here it was envisaged that the reaction of **183** with (trimethylsilyl)methyl lithium would give **182** which on treatment with bromine would yield the α-bromo ketone **181**.

Due to the reputation of α-bromo ketones for being difficult to handle, it was decided to cyclise the ring prior to the asymmetric reduction. It was then hoped that the resulting piperidine ring would produce enough stereoinduction to enable the reduction of **180** to proceed with high stereoselectivity. This would then just leave us with a simple deprotection step in order for us to obtain DNJ.

Since our route to DNJ is based on the same chemistry as that employed in the synthesis of our 5-membered iminosugars, we were optimistic that slight variations in our synthesis would allow for a similar series of analogues to be produced (**Figure 44**).



164 **184** **185**

Figure 44 : Synthetic Analogues of DNJ

Initial efforts focused on the final 7 steps of our synthesis in order to test our new methodology. In an effort to make quick progress we decided to synthesise the racemic C(4)-H derivative **185**, since a large quantity of (±)**98** remained from a previous synthesis within the group (**Figure 45**).

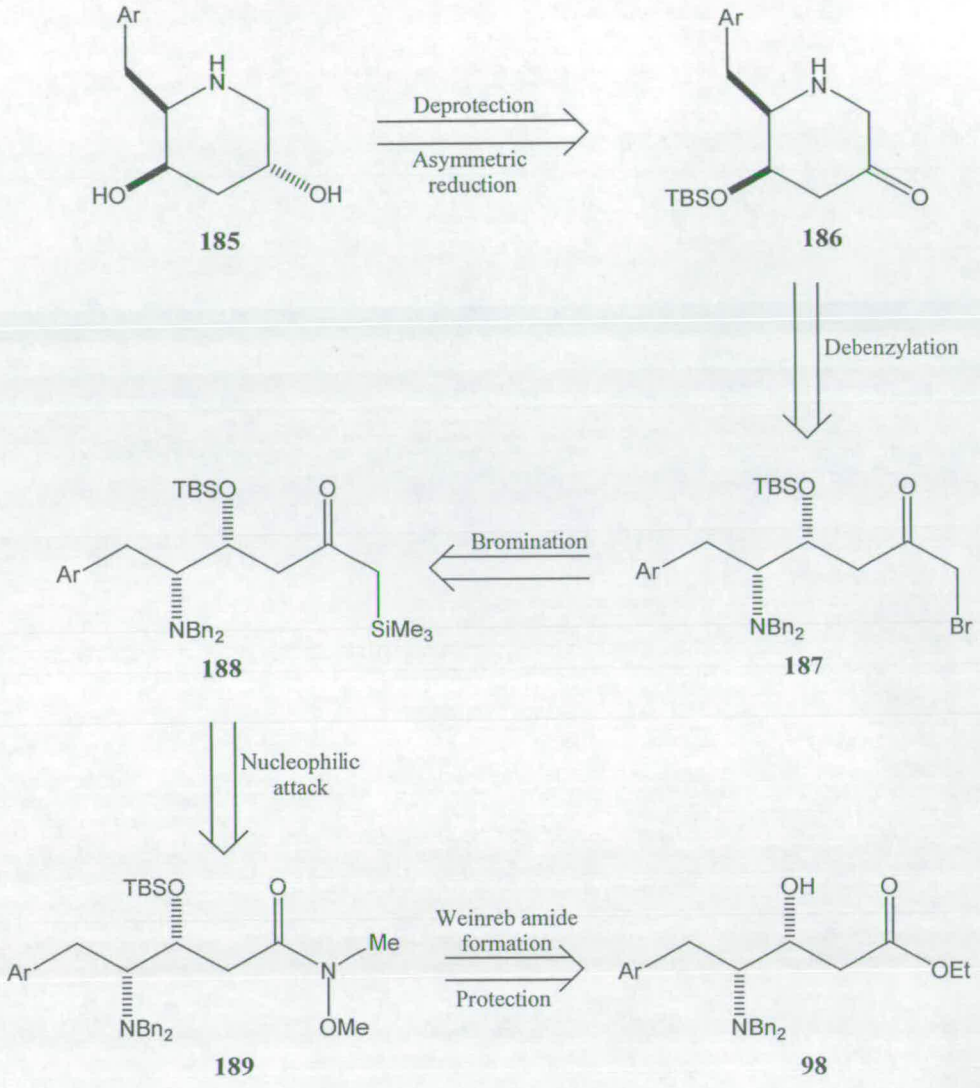
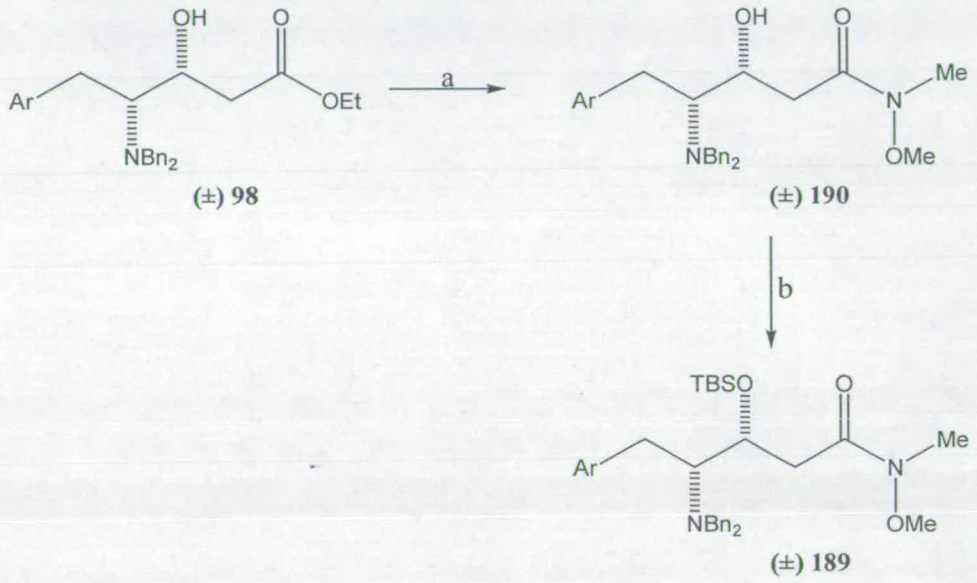


Figure 45 : The Retrosynthesis for (2*R*,3*R*,5*R*)-3,5-Dihydroxy-2-(4-methoxybenzyl)piperidine

5.4 The Synthesis of (2*R*,3*R*,5*R*)-3,5-Dihydroxy-2-(4-methoxybenzyl)piperidine

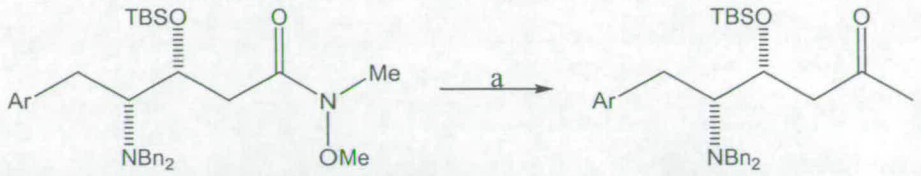
The reduced Claisen product (±)**98** (*cf* chapter 3) was readily converted to its Weinreb amide **190** in 97% yield and subsequent treatment with *tert*-butyldimethylsilyltriflate proceeded smoothly to give the protected alcohol **189** in 84% yield (**Scheme 64**).



Reagents: (a) (MeO)N(Me)H•HCl, $AlMe_3$, THF (97%); (b) TBSOTf, 2,6-lutidine, CH_2Cl_2 (84%).

Scheme 64

Treatment of **189** with (trimethylsilyl)methyl lithium failed to give the desired product, and instead gave the methyl ketone **191** (**Scheme 65**). It was therefore proposed that the acetic acid used in the work-up was promoting desilylation thus resulting in the formation of the methyl ketone.

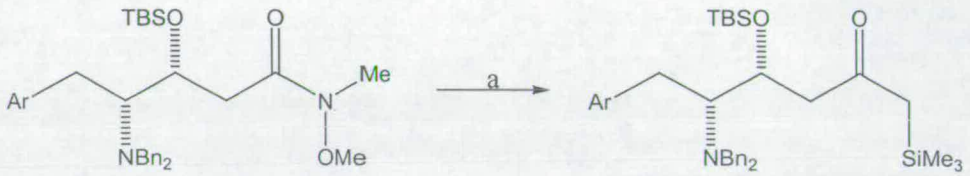


(±) **189** (±) **191**

Reagents: (a) (i) $(CH_3)_3SiCH_2Li$, THF; (ii) 2 M AcOH in THF, pH 7 phosphate buffer (90%).

Scheme 65

In an effort to prevent desilylation re-occurring, the use of acetic acid was removed from the work-up. This had the desired effect, and allowed us to access the trimethylsilylketone **188** in 100% yield (**Scheme 66**). Recent reports in the literature[134] suggested that similar ketones were unstable to silica gel and consequently, in view of our own experiences, it was decided to use the ketone crude in subsequent steps.



(±) **189** (±) **188**

Reagents: (a) (i) $(CH_3)_3SiCH_2Li$, THF; (ii) pH 7 phosphate buffer (100%).

Scheme 66

The reaction of trimethylsilylketone **188** with bromine failed to give the α-bromoketone **187**, and instead gave a mixture of unidentifiable products. Further attempts to obtain the α-bromoketone were not possible due to time constraints. However, it is anticipated that future attempts employing *N*-bromosuccinimide will enable us to access the α-bromoketone.

5.5 Summary of Chapter 5

We have begun looking at the synthesis of 1-deoxynojirimycin with a view towards producing a series of analogues. Our review of previous syntheses indicated that routes starting from natural sugars tended to be short and direct but as a consequence, were often low yielding and did not afford the flexibility required to produce a varied range of analogues.

Our synthesis therefore, is based around the glycolate aldol reaction used to synthesise anisomycin. It is anticipated that by varying reactions and conditions, in a similar way to that shown for anisomycin, it will enable us to access a large range of DNJ analogues.

Chapter 6 : Experimental

6.1 General Experimental

^{1}H nuclear magnetic resonance (NMR) spectra were recorded using an internal deuterium lock for the indicated reference at ambient probe temperatures on Varian Gemini 200 (200 MHz), Bruker AC250 (250 MHz), Bruker AM360 (360 MHz) or Varian Inova 600 (600 MHz) Fourier transform instruments. The data is presented as follows: chemical shift (in ppm on the δ scale relative to δ_{TMS} = 0), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant in hertz and the interpretation. ^{13}C NMR spectra were recorded using an internal deuterium lock for the indicated reference at ambient probe temperatures on Varian Gemini 200 (50.3 MHz), Bruker AC250 (62.9 MHz) or Bruker AM360 (90.6 MHz) instruments. The data is presented as follows: chemical shift (in ppm on the δ scale relative to δ_{TMS} = 0), integration and interpretation (Q = quaternary centre).

Infra-red spectra were recorded on either a Biorad FTS-7, a Perkin Elmer Paragon 1000 FT-IR, or a Jasco FT/IR-410 instrument using 5 mm sodium chloride plates, or KBr discs. The wavelengths of maximum absorbance (ν_{max}) are quoted in cm^{-1}.

Fast atom bombardment (FAB) mass specta were performed on a Kratos MS50TC mass spectrometer. Electron impact (EI) mass spectra were performed on a Finnigan 4500 mass spectrometer. The parent ion or relevant fragment are quoted, followed by significant fragments and their relative intensities.

Optical rotations were measured on an AA-1000 polarimeter or on an Optical Activity PolAAr 20 instrument with a path length of 1.0 dm at the sodium D line (589 nm) and are reported as follows: $[\alpha]_D$, concentration (c in g/100 cm^3), and solvent. All optical rotations were measured at a temperature of 23 °C.

Mps were determined on a Gallenkamp Electrothermal Melting Point apparatus and are uncorrected.

Elemental analysis was carried out on a Perkin Elmer 2400 CHN Elemental analyser. T.l.c. was performed on Merck $60F_{254}$ (0.25 mm) glass backed silica plates and visualised by ultraviolet (UV) light and/or ammonium molybdate stain.[ϕ] Flash column chromatography was carried out on Merck Kieselgel 60 (Merck 9385) under positive pressure by means of a hand pump. Eluent compositions are quoted as v/v ratios.

Chiral high performance liquid chromatography (HPLC) was carried out on a Waters 786 instrument equipped with a Chiracel OD column (internal diameter 4.6 mm) and a UV detector. A standard flow rate of 0.5 cm^3/min was used. Preparative high performance liquid chromatography (HPLC) was carried out on a Gilson instrument equipped with a Spherisorb column (internal diameter 25 mm) and a RI detector. A standard flow rate of 9 cm^3/min was used. All HPLC samples were filtered through 0.45 λm nylon syringe filters prior to analysis. All solvents used for HPLC analysis were vacuum filtered and degassed prior to use.

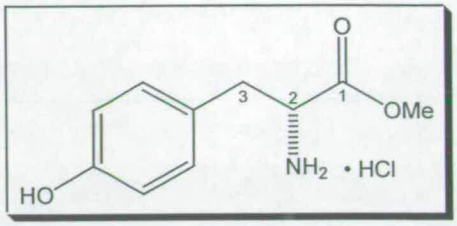
Reagents were purified by standard means.[135] Dichloromethane (DCM), and triethylamine and diisopropylethylamine were distilled from calcium hydride and stored over calcium hydride under an argon atmosphere. Tetrahydrofuran (THF) was distilled from sodium metal/benzophenone ketyl and stored under an argon atmosphere. Acetyl choride, acetic anhydride and propionyl chloride were distilled immediately prior to use. All other reagents were used as supplied.

All experiments were performed in an inert atmosphere of argon under anhydrous conditions using oven dried apparatus cooled in a desiccator or flame

[ϕ] Ammonium molybdate dip prepared as follows: To water (950 cm^3) was added concentrated sulfuric acid (50 cm^3) followed by ammonium molybdate (50 g) and ceric sulfate (3 g). The mixture was stirred until all solid material had disappeared and a bright yellow solution remained.

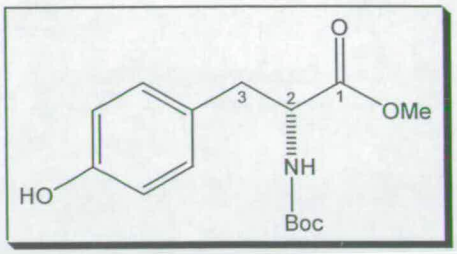
dried under argon prior to use. Standard techniques for the handling of air-sensitive materials were employed.[136]

Methyl (2*R*)-2-amino-3-(4-hydroxyphenyl)propionate hydrochloride salt 37



Acetyl chloride (2.52 g, 2.30 cm^3, 33.0 mmol) was added dropwise to methanol (50 cm^3) at 0 °C. The mixture was stirred for *ca.* 15 min and D-tyrosine (2.00 g, 11.0 mmol) was then added portionwise to the solution. The resulting solution was heated to reflux and held at reflux for 3 hours. Concentration under reduced pressure provided hydrochloride salt **37** (2.55 g, 100%) as a solid. Recrystallisation from methanol provided an analytical sample; mp 191-192 °C; $[\alpha]_D$ -13.1 (*c* 0.73, CH_3OH) [lit.(*ent*-**37**), (Acros) mp 193-194 °C, $[\alpha]_D$ +11.5 (*c* 2, CH_3OH)]; ν_{max} (KBr)/cm^{-1} 3343, 2888, 1745, 1615, 1593; δ_H (200 MHz; CD_3OD) 7.07 (2H, d, *J* 8.6, Ar*H*), 6.78 (2H, d, *J* 8.6, Ar*H*), 4.88 (2H, br s, NH_2), 4.24 (1H, t, *J* 6.6, C_2H), 3.80 (3H, s, O*Me*), 3.19–3.05 (2H, m, $C_3H_XH_Y$ + $C_3H_XH_Y$); δ_c (62.9 MHz; CD_3OD) 168.6 (1C, Q), 156.4 (1C, Q), 129.6 (2C, CH), 123.7 (1C, Q), 114.9 (2C, CH), 53.5 (1C, CH), 51.6 (1C, CH_3), 34.6 (1C, CH_2); (Found: C, 51.33; H, 5.90; N, 5.92. Calc. for $C_{10}H_{13}NO_3$•HCl: C, 51.84; H, 6.05; N, 6.05%).

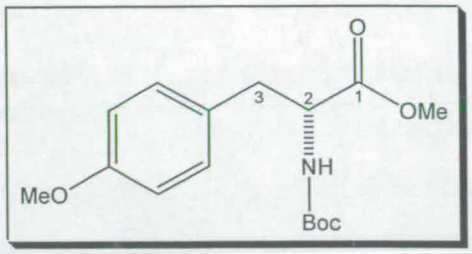
Methyl (2*R*)-2-*tert*-butoxycarbonylamino-3-(4-hydroxyphenyl)propionate 38



To a solution of ester hydrochloride **37** (2.59 g, 11.1 mmol) in ethanol (25 cm^3) was added sodium hydrogen carbonate (2.82 g, 33.5 mmol) and di-*tert*-butoxy carbamate (2.42 g, 11.1 mmol). The resulting solution was stirred for 15 hours and then filtered, and concentrated under reduced pressure to give **38** (3.25 g, 99%) as a solid, R_f [CH_2Cl_2:CH_3OH (95:5)] 0.50; mp 102–104 °C; $[\alpha]_D$ -49.3 (*c* 0.54, $CHCl_3$) [lit.(*ent*-**38**), (Aldrich) mp 100-104 °C, $[\alpha]_D$ +51 (*c* 1, $CHCl_3$)]; ν_{max} (KBr)/cm^{-1} 3358, 2983, 1734, 1687; δ_H (200 MHz; $CDCl_3$) 6.97 (2H, d, *J* 8.4, Ar*H*), 6.72 (2H, d, *J* 8.4, Ar*H*), 5.00 (1H, d, *J* 8.5, N*H*), 4.53 (1H, q, *J* 8.6, C_2H), 3.71 (3H, s, O*Me*), 2.99–3.02 (2H, m, $C_3H_XH_Y$ + $C_3H_XH_Y$), 1.41 (9H, s, ^tBu). δ_c (62.9 MHz; $CDCl_3$) 172.6 (1C, Q), 155.3 (2C, Q), 130.1 (2C, CH), 126.9 (1C, Q), 115.4 (2C, CH), 80.2 (1C, Q), 54.5 (1C, CH), 52.2 (1C, CH_3), 37.3 (1C, CH_2), 28.1 (3C, CH_3); (Found: C, 61.02; H, 7.12; N, 4.75. Calc. for $C_{15}H_{21}NO_5$: C, 61.02; H, 7.11; N, 4.74%).

All spectroscopic data was in good agreement with that of the literature.[137]

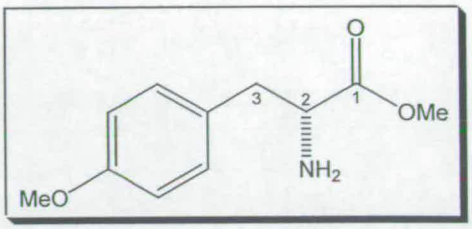
Methyl (2*R*)-2-*tert*-butoxycarbonylamino-3-(4-methoxyphenyl) propionate 39[138]



To a solution of phenol **38** (0.200 g, 0.670 mmol) in DMF (25 cm^3) was added potassium carbonate (0.280 g, 2.03 mmol), and a solution of methyl iodide (0.48 g, 0.21 cm^3, 3.3 mmol) in DMF (2 cm^3). The resulting solution was stirred for 24 hours, and then added to ice. The organic phase was separated off, and the aqueous phase was extracted with EtOAc (3 × 20 cm^3). The combined organic extracts were dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give **39** (0.186 g, 97%) as an oil, R_f [hexane:EtOAc (7:3)] 0.48; $[\alpha]_D$ -52.8 (*c* 1.43, $CHCl_3$) [lit.(*ent*-**39**),[138] $[\alpha]_D$ +59.2 (*c* 1.8, $CHCl_3$)]; ν_{max} (neat)/cm^{-1} 3433, 3364, 1713, 1745, 1612, 1513; δ_H (200 MHz; $CDCl_3$) 7.01 (2H, d, *J* 8.6, Ar*H*), 6.79 (2H, d, *J* 8.6, Ar*H*), 5.03 (1H, d, *J* 8.0, N*H*), 4.50 (1H, q, *J* 3.0, C_2H), 3.73 (3H, s, O*Me*), 3.67 (3H, s, O*Me*), 2.89-3.08 (2H, m, $C_3H_XH_Y$ + $C_3H_XH_Y$), 1.38 (9H, s, ^tBu); δ_C (62.9 MHz; $CDCl_3$) 172.3 (1C, Q), 158.4 (1C, Q), 154.9 (1C, Q), 130.1 (2C, CH), 127.7 (1C, Q), 113.8 (2C, CH), 79.7 (1C, Q), 55.0 (1C, CH_3), 54.4 (1C, CH), 52.1 (1C, CH_3), 37.3 (1C, CH_2), 28.1 (3C, CH_3); *m/z* (FAB) 310 ($[M + H]^+$, 12%), 254 (57), 210 (72), 192 (53), 150 (63), 121 (82); HRMS (FAB) (Found: $[M + H]^+$, 310.1654. $C_{16}H_{24}NO_5$ requires *m/z*, 310.1654).

All spectroscopic data was in good agreement with that of the literature.[138]

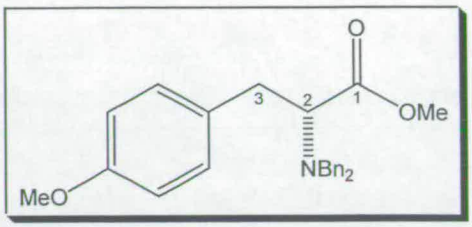
Methyl (2*R*)-2-amino-3-(4-methoxyphenyl)propionate 36



To a solution of Boc-protected ester **39** (0.290 g, 0.939 mmol) in CH_2Cl_2 (10 cm^3) was added trifluoroacetic acid (1.39 g, 0.930 cm^3, 12.2 mmol). The solution turned pink immediately and was stirred for 2 hours. The reaction was quenched by the addition of a saturated solution of sodium carbonate until the solution was rendered alkaline as judged by litmus paper. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3). The combined organic extracts were dried and concentrated under reduced pressure to give **36** (0.194 g, 100%) as an oil, R_f [hexane:EtOAc (1:1)] 0.1; $[\alpha]_D$ -7.76 (*c* 1.26, $CHCl_3$) [lit.(*ent*-**36**),[94] $[\alpha]_D$ +11 (*c* 0.15, $CHCl_3$)]; ν_{max} (neat)/cm^{-1} 3374, 2951, 1735, 1611, 1513; δ_H (200 MHz; $CDCl_3$) 7.01 (2H, d, *J* 8.6, Ar*H*), 6.75 (2H, d, *J* 8.6, Ar*H*), 3.69 (3H, s, O*Me*), 3.62 (3H, s, O*Me*), 3.62–3.56 (1H, m, C_2H), 3.69–3.66 (1H, dd, *J* 13.6, 5.3, $C_3H_XH_Y$), 2.72 (1H, dd, *J* 13.6, 7.7, $C_3H_XH_Y$), 1.46 (2H, brs, NH_2); δ_C (62.9 MHz; $CDCl_3$) 175.3 (1C, Q), 158.3 (1C, Q), 130.1, (2C, CH) 128.8 (1C, Q), 113.8 (2C, CH), 55.7 (1C, CH), 55.0 (1C, CH_3), 51.8 (1C, CH_3), 39.9 (1C, CH_2); *m/z* (FAB) 210 ($[M + H]^+$, 100%), 193 (29), 150 (56), 121 (58); HRMS (FAB) (Found: $[M + H]^+$, 210.1130. $C_{11}H_{16}NO_3$ requires *m/z*, 210.1130).

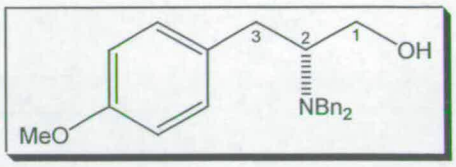
All spectroscopic data was in good agreement with that of the literature.[94, 139]

Methyl (2*R*)-2-dibenzylamino-3-(4-methoxyphenyl)propionate 41



To a solution of amino ester **36** (6.55 g, 31.3 mmol) in acetonitrile (40 cm^3) was added potassium carbonate (21.6 g, 157 mmol) followed by benzyl bromide (21.4 g, 14.9 cm^3, 125 mmol). The mixture was stirred for 48 hours. Water (30 cm^3) was added and the aqueous phase was extracted with EtOAc (3 × 75 cm^3). The combined organic extracts were dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (4 : 1)] to give **41** (11.59 g, 95%) as an oil, R_f [hexane:EtOAc (7:3)] 0.66; $[\alpha]_D$ +73.25 (*c* 1.12, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3028, 2834, 1731, 1612, 1581, 1513; δ_H (200 MHz; $CDCl_3$) 7.32–7.16 (10H, m, Ar*H*), 6.94 (2H, d, *J* 8.7, Ar*H*), 6.78 (2H, d, *J* 8.7, Ar*H*), 3.96 (2H, d, *J* 13.9, NCH_XH$_Y$Ph × 2), 3.81 (3H, s, O*Me*), 3.73 (3H, s, O*Me*), 3.63 (1H, t, *J* 7.9, C_2H), 3.54 (2H, d, *J* 13.9, NCH$_X$$H_Y$Ph × 2), 3.08 (1H, dd, *J* 13.9, 7.9, $C_3H_AH_B$), 2.93 (1H, dd, *J* 13.9, 7.9, $C_3H_AH_B$); δ_C (50.3 MHz; $CDCl_3$) 172.7 (1C, Q), 158.0 (1C, Q), 139.2 (3C, Q), 130.1 (2C, CH), 128.6 (4C, CH), 128.0 (4C, CH), 126.8 (2C, CH), 113.5 (2C, CH), 62.4 (1C, CH), 55.2 (1C, CH_3), 54.3 (2C, CH_2) 51.0 (1C, CH_3), 34.8 (1C, CH_2); *m/z* (FAB) 390 ($[M + H]^+$, 33%), 330 (15), 268 (40), 121 (15), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 390.2069. $C_{25}H_{28}NO_3$ requires *m/z,* 390.2069).

(2*R*)-2-Dibenzylamino-3-(4-methoxyphenyl)propan-1-ol 42

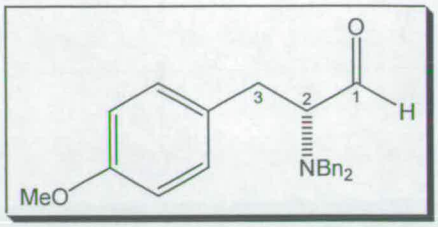


To a solution of ester **41** (12.2 g, 31.4 mmol) in ether (40 cm^3) was added methanol (5.1 cm^3) followed by lithium borohydride (2.73 g, 125 mmol). The solution was heated to reflux and held at reflux for 4 hours. The reaction was quenched by the addition of saturated aq. ammonium chloride (50 cm^3). The organic phase was then separated, and the aqueous phase was extracted with EtOAc (3 × 75 cm^3). The combined organics were washed with brine (2 × 150 cm^3) then dried, and concentrated under reduced pressure to give a solid, which was recrystallised from toluene to give **42** (9.86 g, 87%) as a solid, R_f [hexane:EtOAc (7:3)] 0.45; mp 112–113 °C; $[\alpha]_D$ -54.4 (*c* 2.00, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3332, 3024, 2923, 1611, 1512; δ_H (200 MHz; $CDCl_3$) 7.40–7.22 (10H, m, Ar*H*), 7.04 (2H, d, *J* 8.8, Ar*H*), 6.84 (2H, d, *J* 8.8, Ar*H*), 3.94 (2H, d, *J* 13.2, NCH_XH_YPh × 2), 3.80 (3H, s, O*Me*), 3.57-3.44 (1H, m, CH_AH_BOH), 3.50 (2H, d, *J* 13.2, NCH_XH_Y × 2), 3.35 (1H, dd, *J* 10.3, *J* 4.4, CH_AH_BOH), 3.12–2.98 (3H, m, C_2H + $C_3H_XH_Y$ + $C_3H_XH_Y$), 2.47–2.34 (1H, m, CH_AH_BOH); δ_c (62.9 MHz; $CDCl_3$) 157.9 (1C, Q), 139.0 (2C, Q), 130.9 (1C, Q), 129.7 (2C, CH), 128.9 (4C, CH), 128.4 (4C, CH), 127.1 (2C, CH), 113.8 (2C, CH), 60.7 (1C, CH), 60.2 (1C, CH_2), 55.1 (1C, CH_3), 53.0 (2C, CH_2), 30.6 (1C, CH_2); (Found: C, 79.62; H, 7.36; N, 3.85. $C_{24}H_{27}NO_2$ Calc. for $C_{24}H_{27}NO_2$: C, 79.70; H, 7.50; N, 3.90%).

Via reduction of aldehyde **31**.

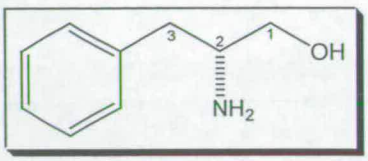
To a solution of aldehyde **31** (see below), (0.192 g, 0.535 mmol) in toluene (3 cm^3) at -78 °C was added diisobutylaluminium hydride [2.23 cm^3 (1.0 M in hexanes), 2.23 mmol]. The mixture was stirred at -78 °C for 5 min, then quenched by sequential addition of water (0.050 cm^3), aq. Sodium hydroxide (50 mm^3; 1 M), and water (10 cm^3). The resulting mixture was allowed to warm to room temperature and extracted with CH_2Cl_2 (3 × 25 cm^3). The combined organics were dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give alcohol **42** (0.173 g, 90%) as a solid, chiral HPLC (*S* enantiomer) R_t = 19.4 minutes, (*R* enantiomer) R_t = 22.7 minutes [hexane-propan-2-ol (9 : 1)], >98% ee.

(2*R*)-2-Dibenzylamino-3-(4-methoxyphenyl)propanal 31



To a solution of oxalyl chloride (0.17 g, 0.12 cm^3, 1.3 mmol) in CH_2Cl_2 (10 cm^3) at -78 °C was added dropwise a solution of DMSO (0.13 g, 0.12 cm^3, 1.7 mmol) in CH_2Cl_2 (0.5 cm^3). The mixture was stirred for *ca.* 5 min whereupon it became cloudy. A solution of alcohol **42** (0.200 g, 0.60 mmol) in CH_2Cl_2 (3.0 cm^3) at -78 °C was introduced *via* cannula. The resulting clear solution was stirred at -78 °C for 1 hour. Triethylamine (0.72 g, 1.0 cm^3, 7.0 mmol) was added and the resulting cloudy solution was allowed to warm to room temperature. Water (10 cm^3) was added producing two clear phases. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3). The combined organics were washed sequentially with 1% HCl (20 cm^3), water (20 cm^3), saturated aq. sodium bicarbonate (20 cm^3) and brine (20 cm^3), then dried and concentrated under reduced pressure to give aldehyde **31** (0.198 g, 100%) as an oil which was a used in the aldol reaction without further purification, R_f [hexane:EtOAc (7:3)] 0.69; $[\alpha]_D$ +70.3 (*c* 2.00, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3026, 2833, 2716, 1727, 1611, 1583, 1512; δ_H (200 MHz; $CDCl_3$) 9.61 (1H, s, C*H*O), 7.22–7.05 (10H, m, Ar*H*), 6.96 (2H, d, *J* 8.8, Ar*H*), 6.71 (2H, d, *J* 8.8, Ar*H*), 3.73 (2H, d, *J* 13.8, NCH_AH_BPh × 2), 3.68 (3H, s, O*Me*), 3.57 (2H, d, *J* 13.8, NCH_AH_BPh × 2), 3.41 (1H, t, *J* 6.8, C_2H), 2.99 (1H, dd, *J* 14.0, 6.8, $C_3H_XH_Y$), 2.78 (1H, dd, *J* 14.0, 6.8, $C_3H_XH_Y$); δ_c (69.2 MHz; $CDCl_3$) 202.2 (1C, CH), 157.9 (1C, Q), 138.8 (2C, Q), 130.8 (1C, Q), 130.2 (2C, CH), 128.6 (4C, CH), 128.2 (4C, CH), 127.2 (2C, CH), 113.7 (2C, CH), 68.4 (1C, CH), 55.1 (1C, CH_3), 54.6 (2C, CH_2), 29.0 (1C, CH_2); m/z (EI) 360.0 (M^+, 9%), 238.7 (8), 197.6 (100).

(2*R*)-2-Amino-3-phenylpropan-1-ol 44



Method A:

To a solution of D-phenylanaline (10.0 g, 60.6 mmol) in THF (200 cm^3) at 0 °C was added sodium borohydride (5.52 g, 146 mmol), and a solution of iodine (15.3 g, 60.6 mmol) in THF (30 cm^3). The reaction was heated to reflux and held there for 24 hours, and then re-cooled to 0 °C and quenched, carefully, by the addition of methanol (20 cm^3). The solution was stirred at room temperature for a further 30 minutes. The mixture was then concentrated under reduced pressure before being dissolved in a 20% aqueous potassium hydroxide solution (200 cm^3) and then allowed to stir for a further 18 hours. The resulting mixture was extracted with CH_2Cl_2 (3 × 75 cm^3), and the combined organic phase was dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [CH_2Cl_2:CH_3OH (95:5)] to give **44** (5.45g, 60%) as a white solid. R_f [hexane:EtOAc (1:1)] 0.29; mp 87–88 °C; $[\alpha]_D$ +22.2 (*c* 1.3, EtOH) [lit.,[140] mp 88–89 °C, $[\alpha]_D$ +22.1 (*c* 1.5, EtOH)]; ν_{max} (KBr)/cm^{-1} 3422, 3357, 2876, 1577, 1493; δ_H (200 MHz; $CDCl_3$) 7.35–7.09 (5H, m, Ar*H*), 3.62 (1H, dd, *J* 10.7, 3.9, $C_1H_AH_B$), 3.38 (1H, dd, *J* 10.7, 7.0, $C_1H_AH_B$), 3.10 (1H, dddd, *J* 8.6, 7.0, 5.1, 3.9, C_2H), 2.78 (1H, dd, *J* 13.6, 5.1, $C_3H_XH_Y$), 2.50 (1H, dd, *J* 13.6, 8.6, $C_3H_XH_Y$), 2.27 (2H, br s, NH_2); δ_c (62.9 MHz; $CDCl_3$) 138.5 (1C, Q), 129.0 (2C, CH), 128.4 (2C, CH), 126.2 (1C, CH), 65.9 (1C, CH_2), 54.0 (1C, CH), 40.5 (1C, CH_2); (Found: C, 70.83; H, 8.66; N, 9.18. $C_9H_{13}NO$ requires C, 71.52; H, 8.61; N, 9.27%).

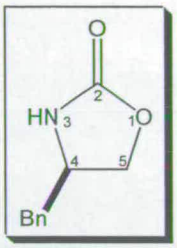
All spectroscopic data was in good agreement with that of the literature.[140]

Method B[137]

To a solution of lithium borohydride (2.67 g, 118 mmol) in THF (50 cm^3) at 0 °C was added trimethylsilyl chloride (26.3 g, 30.9 cm^3, 242 mmol). The mixture was warmed to room temperature and allowed to stir for 20 minutes before being recooled to 0 °C. D-phenylanaline (10.0 g, 60.6 mmol) was added and the solution was warmed to room temperature and left to stir for 24 hours. The solution was again recooled and quenched by the dropwise addition of methanol (50 cm^3), followed by 2.5 M aqueous sodium hydroxide (50 cm^3). The organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ (3 × 100 cm^3). The combined organic extracts were dried and concentrated under reduced pressure to give a solid which was recrystallised from toluene to give **44** (8.65 g, 95%) as a white solid, mp 86–88 °C.

All spectroscopic data was identical to the compound synthesised above.

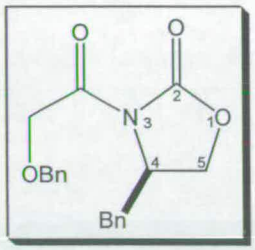
(4*R*)-4-Phenylmethyloxazolidin-2-one 45



To a flask containing D-phenylalaninol (5.00 g, 33.1 mmol) was added diethyl carbonate (8.01 cm^3, 7.81 g, 66.2 mmol) and potassium carbonate (0.457 g, 3.31 mmol). The mixture was heated to carefully to 135–140 °C, and the ethanol produced was allowed to distil off as it was formed (over *ca* 2 hours). The mixture was cooled to room temperature and diluted with CH_2Cl_2 (75 cm^3) before being filtered to remove any of the remaining potassium carbonate. The organic phase was washed with saturated aq. sodium bicarbonate (50 cm^3) then dried, and concentrated under reduced pressure to give a solid which was recrystallised from [hexane:EtOAc] to give **45** (4.87 g, 83%) as a white solid, R_f [hexane:EtOAc (1:1)] 0.29; mp 87–88 °C; $[\alpha]_D$ –4.38 (*c* 1.1, EtOH) [lit.,[141] mp 86–88 °C; $[\alpha]_D$ –4.58 (*c* 1.1, EtOH)]; ν_{max} (KBr)/cm^{-1} 3282, 2924, 1752, 1709, 1548, 1496; δ_H (250 MHz; $CDCl_3$) 7.36–7.15 (5H, m, Ar*H*), 6.11 (1H, br s, N*H*), 4.44–4.36 (1H, m, C_4H), 4.09 (1H, dd, *J* 12.7, 5.4, $C_5H_AH_B$), 4.15–4.02 (1H, m, $C_5H_AH_B$), 2.90 (1H, dd, *J* 13.3, 6.5, CH_XH_YPh), 2.82 (1H, dd, *J* 13.3, 6.2, CH_XH_YPh); δ_c (62.9 MHz; $CDCl_3$) 159.5 (1C, Q), 135.7 (1C, Q), 128.9 (2C, CH), 128.8 (2C, CH), 127.0 (1C, CH), 69.4 (1C, CH_2), 53.6 (1C, CH), 41.2 (1C, CH_2); (Found: C, 68.03; H, 6.05; N, 7.74. $C_{10}H_{11}NO_2$ requires C, 67.80; H, 6.21; N, 7.91%).

All spectroscopic data was in good agreement with that of the literature.[141]

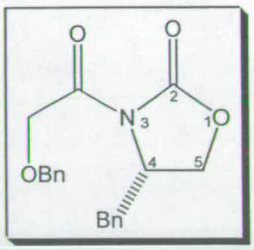
(4*R*)-3-(2'-Benzyloxy-1'-oxoethyl)-4-phenylmethyloxazolidin-2-one 32



To a solution of oxazolidinone **45** (2.00 g, 11.3 mmol) in THF (50 cm^3) at -78 °C was added *n*-butyllithium (7.29 cm^3, 1.55 M in hexanes, 11.3 mmol) and the solution turned orange. The solution was allowed to stir for 15 minutes before benzyloxyacetyl chloride (1.78 cm^3, 2.08 g, 11.3 mmol) was added causing the orange colour to disappear. The mixture was left to stir for 1 hour and was then quenched by the addition of a saturated aqueous solution of ammonium chloride (50 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 cm^3); the combined organic phase was washed sequentially with saturated aq. sodium bicarbonate (75 cm^3) and brine (75 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (4:1)] to give **32** (3.27 g, 89%) as a white solid, R_f [hexane:EtOAc (7:3)] 0.37; mp 69–70 °C; $[\alpha]_D$ -57.4 (*c* 1.52, $CHCl_3$) [lit.,[97(a)] mp 67–69 °C, $[\alpha]_D$ -57.45 (*c* 2.0, $CHCl_3$)]; ν_{max} (KBr)/cm^{-1} 3031, 2909, 2841, 1766, 1708, 1603, 1497; δ_H (200 MHz; $CDCl_3$) 7.45–7.18 (10H, m, Ar*H*), 4.80–4.62 (5H, m, CH_2OBn + OCH_2Ph + C_4H), 4.27 (1H, t, *J* 9.2, $C_5H_AH_B$), 4.21 (1H, dd, *J* 9.2, 3.7, $C_5H_AH_B$), 3.33 (1H, dd, *J* 13.2, 3.3, CH_XH_YPh), 2.82 (1H, dd, *J* 13.2, 9.2, CH_XH_YPh); δ_c (62.9 MHz; $CDCl_3$) 170.0 (1C, Q), 153.2 (1C, Q), 137.0 (1C, Q), 134.8 (1C, Q), 129.3 (2C, CH), 128.9 (2C, CH), 128.4 (2C, CH), 127.9 (2C, CH), 127.8 (1C, CH), 127.3 (1C, CH), 73.8 (1C, CH_2), 69.5 (1C, CH_2), 67.1 (1C, CH_2), 54.6 (1C, CH), 37.6 (1C, CH_2).

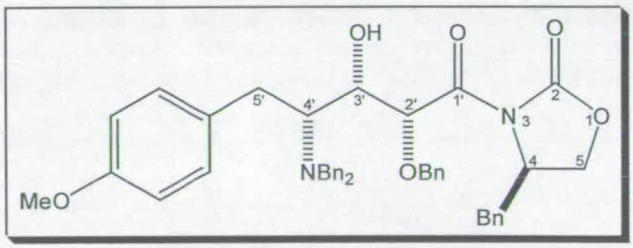
All spectroscopic data was in good agreement with that of the literature.[97(a)]

(4*S*)-3-(2'-Benzyloxy-1'-oxoethyl)-4-phenylmethyloxazolidin-2-one *ent*-32



Synthesised in analogous manner to the *R*-enantiomer from *ent*-**45**. Thus, to oxazolidinone *ent*-**45** (2.50 g, 14.2 mmol) in THF (50 cm^3) at -78 °C was added *n*-butyllithium (9.16 cm^3, 1.55 M in hexanes, 14.2 mmol) followed by benzyloxyacetyl chloride (2.24 cm^3, 2.62 g, 14.2 mmol) to give *ent*-**42** (4.19 g, 92%) as a white solid, mp 68–70 °C; $[\alpha]_D$ +57.1 (*c* 1.72, $CHCl_3$) [lit.,[112(g)] mp 67–69 °C, $[\alpha]_D$ +56.0 (*c* 1.71, $CHCl_3$)].

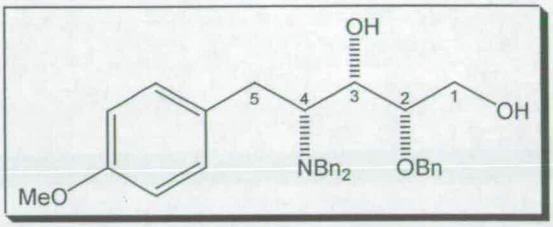
(2'*R*,3'*S*,4*R*,4'*R*)-3-[2'-Benzyloxy-4'-*N*,*N*-dibenzylamino-3'-hydroxy-5'-(4''-methoxyphenyl)-1'-oxopentyl]-4-phenylmethyloxazolidin-2-one 30



To a solution of glycolate equivalent **32** (0.66 g, 2.0 mmol) in CH_2Cl_2 (5 cm^3) at -78 °C was added triethylamine (0.27 g, 0.37 cm^3, 2.7 mmol) followed by dropwise addition of dibutylboron triflate (1.0 M in CH_2Cl_2; 2.5 cm^3, 2.5 mmol). The solution was stirred at -78 °C for 45 minutes, then allowed to warm to 0 °C over 30 minutes and stirred at 0 °C for 1.25 hours. The solution was then recooled to -78 °C and a -78 °C solution of aldehyde **31** (0.200 g, 0.557 mmol) in CH_2Cl_2 (3 cm^3) was added dropwise *via* cannula. The reaction mixture was stirred at -78 °C for 1 h, and allowed

to warm to 0 °C over a period of 4 hours. The reaction was quenched by the addition of methanol (8 cm^3) followed by pH 7 phosphate buffer (5 cm^3). Hydrogen peroxide (30% aq. solution; 5 cm^3 in methanol (5 cm^3) was added dropwise to the solution and the mixture was stirred and warmed to room temperature over *ca.* 1 hour. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was washed sequentially with saturated aq. sodium bicarbonate (25 cm^3) and brine (25 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give **30** (0.286 g, 75%) as a foam, R_f [hexane:EtOAc (7:3)] 0.42; $[\alpha]_D$ -47.0 (*c* 2.75, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3334, 3027, 2930, 1771, 1709, 1611, 1512; δ_H (200 MHz; $CDCl_3$) 7.38–6.86 (20H, m, Ar*H*), 7.08 (2H, d, *J* 8.6, Ar*H*), 6.66 (2H, d, *J* 8.6, Ar*H*), 4.89 (1H, d, *J* 1.6, C_2·*H*), 4.67 (1H, dddd, *J* 9.9, 6.8, 3.1, 2.5, C_4*H*), 4.52 (1H, s, O*H*), 4.25 (1H, dd, *J* 8.8, 6.8, $C_5H_AH_B$), 4.24 (1H, d, *J* 11.4, OCH_SH_TPh), 4.20–4.16 (1H, m, $C_5H_AH_B$), 4.06 (1H, dd, *J* 9.5, 1.6, C_3·*H*), 3.91 (2H, d, *J* 13.2, NCH_XH_YPh × 2), 3.69 (3H, s, O*Me*), 3.58 (1H, ddd, *J* 9.5, 7.7, 4.6, C_4·*H*), 3.46 (2H, d, *J* 13.2, NCH_XH_YPh × 2), 3.31 (1H, dd, *J* 13.4, 3.1, $C_4HCH_MH_NPh$), 3.30 (1H, d, *J* 11.4, OCH_SH_TPh), 3.14 (1H, dd, *J* 14.6, 4.6, C_5·H_EH_F), 2.74 (1H, dd, *J* 13.4, 9.9, $C_4HCH_MH_NPh$), 2.72 (1H, dd, *J* 14.6, 7.7, C_5·H_EH_F); δ_C (62.9 MHz; $CDCl_3$) 170.0 (1C, Q), 157.7 (1C, Q), 153.4 (1C, Q), 138.3 (2C, Q), 137.5 (1C, Q), 135.1 (1C, Q), 131.7 (1C, Q), 130.1 (2C, CH), 129.2 (2C, CH), 128.8 (5C, CH), 128.7 (3C, CH), 128.4 (4C, CH), 127.7 (2C, CH), 127.1 (2C, CH), 126.7 (2C, CH), 113.5 (2C, CH), 76.9 (1C, CH), 71.1 (1C, CH), 70.9 (1C, CH_2), 66.9 (1C, CH_2), 59.9 (1C, CH), 55.7 (1C, CH_3), 55.0 (1C, CH), 37.5 (2C, CH_2), 30.1 (2C, CH_2); *m/z* (FAB) 685 ($[M + H]^+$, 5%), 307 (28), 289 (16), 154 (100), 136 (93); HRMS (FAB) (Found: $[M + H]^+$, 685.3260. $C_{43}H_{45}N_2O_6$ requires *m/z,* 685.3278).

(2*S*,3*S*,4*R*)-Benzyloxy-4-*N*,*N*,-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentan-1-ol 29



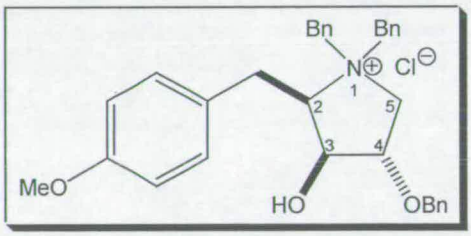
To a solution of aldol adduct **30** (0.148 g, 0.216 mmol) in THF (10 cm^3) at 0 °C was added methanol (0.040 cm^3, 1.1 mmol) and $LiBH_4$ (0.024 g, 1.1 mmol). The solution was warmed to room temperature and stirred for 18 hours then re-cooled to 0 °C and quenched by the addition of 1 M sodium hydroxide (1 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was washed with brine (30 cm^3), dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give the diol **29** (0.088 g, 80%) as a foam, R_f [hexane:EtOAc (7:3)] 0.18; $[\alpha]_D$ -29.6 (*c* 1.1, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3344, 2927, 1609, 1510; δ_H (360 MHz; $CDCl_3$) 7.37–6.96 (15H, m, Ar*H*), 7.04 (2H, d, *J* 8.7, Ar*H*), 6.73 (2H, d, *J* 8.7, Ar*H*), 4.46 (1H, d, *J* 11.8, OCH_AH_BPh), 3.96 (2H, d, *J* 14.5, NCH_XH_YPh × 2), 3.90 (1H, d, *J* 11.8, OCH_AH_BPh), 3.74 (3H, s, O*Me*), 3.72 (1H, dd, *J* 8.1, 3.5, C_3H), 3.69 (1H, dd, *J* 11.7, 4.8, CH_SH_TOH), 3.52 (1H, dd, *J* 11.7, 3.8, CH_SH_TOH), 3.42 (2H, d, *J* 14.5, NCH_XH_YPh × 2), 3.40–3.31 (2H, m, C_2H + C_4H), 3.08 (1H, dd, *J* 14.1, 5.8, $C_5H_MH_N$), 2.70 (1H, dd, *J* 14.1, 7.2, $C_5H_MH_N$), 2.52 (1H, br s, O*H*), 1.78 (1H, br s, O*H*); δ_C (62.9 MHz; $CDCl_3$) 157.9 (1C, Q), 138.7 (1C, Q), 138.2 (1C, Q), 131.6 (1C, Q), 130.0 (2C, CH), 129.0 (4C, CH), 128.4 (4C, CH), 128.1 (2C, CH), 127.2 (2C, CH), 127.1 (2C, CH), 127.0 (2C, CH + Q), 113.8 (2C, CH), 77.6 (1C, CH), 72.1 (1C, CH), 70.9 (1C, CH_2), 61.6 (1C, CH_2), 59.0 (1C, CH), 55.1 (1C, CH_3), 54.0 (2C, CH_2), 31.0 (1C, CH_2); *m/z* (FAB) 512 ($[M + H]^+$, 25%), 330 (21), 154 (24), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 512.2801. $C_{33}H_{38}NO_4$ requires *m/z*, 512.2801).

*Via reduction of methyl ester **150**.*

A solution of methyl ester **150** (0.0168 g, 0.0312 mmol) in THF (6 cm^3) was placed at -78 °C and lithium aluminium hydride (0.15 cm^3, 1.0 M in THF, 0.15 mmol) was added. The solution was slowly warmed to room temperature and stirred for 18 hours before being quenched by the addition of 1 M aqueous sodium hydroxide (2 cm^3). The mixture was diluted with CH_2Cl_2 (10 cm^3) and saturated aq. sodium potassium tartrate (20 cm^3) was added. The biphasic mixture was stirred vigorously for 15 hours by which time two clear phases were apparent. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3). The combined organics were dried, and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give **29** (0.0115 g, 72%) as an oil.

All spectroscopic data was identical to that obtained for the compound above.

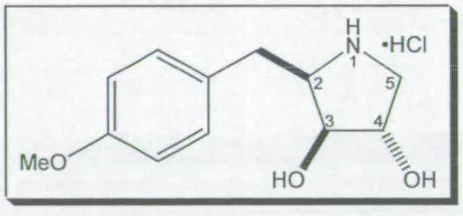
(2*R*,3*S*,4*S*)-4-benzyloxy-1,1-dibenzyl-3-hydroxy-2-(4-methoxybenzyl)pyrrolidinium chloride 48



To a solution of the alcohol **29** (0.110 g, 0.215 mmol) in CH_2Cl_2 (5 cm^3) at 0 °C was added DMAP (0.118 g, 0.967 mmol) and toluene sulfonyl chloride (TsCl) (0.123 g, 0.646 mmol). The solution was stirred for 18 hours then diluted with CH_2Cl_2 (20 cm^3) and water (25 cm^3). The organic phase was separated and washed with 1% HCl (2 × 25 cm^3) then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [CH_2Cl_2:MeOH (100:0) → (95:5)] to give a white foam. The salt obtained was subjected to ion exchange chromatography [Dowex Cl^-,

(prepared by treating Dowex 1-X2 with 1% aqueous hydrochloric acid followed by flushing with methanol until the eluent returned to pH 7)] eluting with methanol to give the chloride salt **48** (0.096 g, 85%) as a foam, R_f [CH_2Cl_2:MeOH (95:5)] 0.05; $[\alpha]_D$ +4.1 (*c* 0.8, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3147, 1612, 1513; δ_H (360 MHz; $CDCl_3$) 7.73–6.71 (17H, m, Ar*H*), 6.69 (2H, d, *J* 8.7, Ar*H*), 5.66 (1H, d, *J* 13.5, OCH_AH_BPh), 5.04 (2H, s, $NCH_XH_YPh \times 2$), 4.71 (1H, br s C_4H), 4.42 (2H, s, $NCH_XH_YPh \times 2$), 4.26 (1H, s, C_3H), 4.26–4.18 (1H, m, $C_5H_SH_T$), 4.20 (1H, d, *J* 13.5, OCH_AH_BPh), 3.82 (1H, dt, *J* 12.2, 2.9, C_2H), 3.69 (3H, s, O*Me*), 3.66 (1H, br d, *J* 12.2, CH_MH_NAr), 3.50 (1H, t, *J* 12.2, CH_MH_NAr), 3.09 (1H, dd, *J* 13.3, 3.1, $C_5H_SH_T$), 2.18 (1H, brs, O*H*); δ_C (62.9 MHz; $CDCl_3$) 158.5 (1C, Q), 137.1 (1C, Q), 133.5 (2C, CH), 133.0 (2C, CH), 130.7 (1C, CH), 130.5 (2C, CH), 129.3 (3C, CH), 128.1 (3C, CH), 128.0 (1C, Q), 127.5 (4C, CH), 127.4 (1C, Q), 126.9 (1C, Q), 114.1 (2C, CH), 80.2 (1C, CH), 75.1 (1C, CH), 72.3 (1C, CH), 71.8 (1C, CH_2), 63.8 (1C, CH_2), 63.5 (1C, CH_2), 62.2 (1C, CH_2), 55.0 (1C, CH_3), 27.7 (1C, CH_2); *m/z* (FAB) 494 ($[M]^+$, 75%), 282 (11), 154 (43), 136 (32), 121 (14), 91 (100); HRMS (FAB) (Found: $[M]^+$, 494.2695. $[C_{33}H_{36}NO_3]^+Cl^-$ requires *m/z*, 494.2695).

(2*R*,3*S*,4*S*)-3,4-Dihydroxy-2-(4-methoxybenzyl)pyrrolidine hydrochloride 54

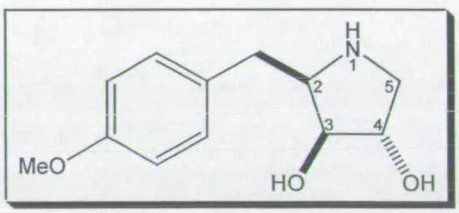


A solution of the chloride salt **48** (0.112 g, 0.212 mmol) and Pearlman's catalyst [0.112 g; 20% $Pd(OH)_2/C$] in methanol (5 cm^3) was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 24 hours. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give **54** (0.055 g, 100%) as a white solid, mp 225–227 °C; $[\alpha]_D$ +8.1 (*c* 0.55, MeOH) [lit.,[4]

mp 224–226 °C]; ν_{max} (KBr)/cm^{-1} 3358, 3252, 2950, 1613, 1585, 1513; δ_H (250 MHz; $CDCl_3$) 7.36 (2H, d, *J* 8.8, Ar*H*), 7.00 (2H, d, *J* 8.8, Ar*H*), 4.34 (1H, dd, *J* 4.2, 1.4, C_4H), 4.07 (1H, dd, *J* 2.8, 1.4, C_3H), 3.87 (3H, s, O*Me*), 3.96 (1H, ddd, *J* 8.3, 7.1, 2.8, C_2H), 3.70 (1H, dd, *J* 12.5, 4.2, $C_5H_AH_B$), 3.24 (1H, dd, *J* 14.1, 7.1, CH_XH_YAr), 3.18 (1H, d, *J* 12.5, $C_5H_AH_B$), 3.04 (1H, dd, *J* 14.1, 8.3, CH_XH_YAr); δ_C (62.9 MHz; $CDCl_3$) 158.5 (1C, Q), 129.2 (2C, CH), 128.0 (1C, Q), 113.4 (2C, CH), 74.1 (1C, CH), 73.8 (1C, CH), 63.4 (1C, CH), 53.8 (1C, CH_3), 50.6 (1C, CH_2), 30.2 (1C, CH_2); *m/z* (FAB) 224 ([M + H]$^+$, 100%), 206 (13), 150 (11), 121 (54), 91 (58); HRMS (FAB) (Found: [M + H]$^+$, 224.1284. $[C_{12}H_{18}NO_3]^+Cl^-$ requires *m/z*, 224.1287).

All spectroscopic data was in good agreement with that of the literature.[4]

(2*R*,3*S*,4*S*)-3,4-Dihydroxy-2-(4-methoxybenzyl)pyrrolidine 14

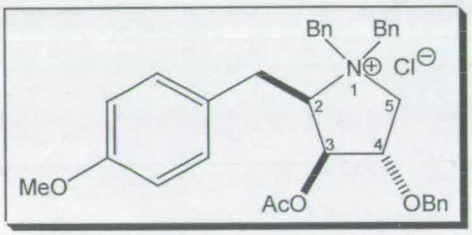


The HCl salt **54** was subjected to ion-exchange chromatography [Dowex OH$^-$] (prepared by treating Dowex 1-X2 with 1 M aqueous NaOH, followed by methanol until the eluent returned to pH 7). Eluting with methanol gave the free base (quantitative recovery) as a white solid, mp 173–175 °C; $[\alpha]_D$ -19.8 (*c* 1.0, MeOH) [lit.,[4] mp 176–178 °C, $[\alpha]_D$ -20 (*c* 1.0, MeOH)]; ν_{max} (KBr)/cm^{-1} 3400, 3271, 2916, 1612, 1512; δ_H (250 MHz; $CDCl_3$) 7.29 (2H, d, *J* 8.8, Ar*H*), 6.92 (2H, d, *J* 8.8, Ar*H*), 4.16 (1H, dd, *J* 3.8, 1.2, C_3H), 3.85 (3H, s, O*Me*), 3.79 (1H, dt, *J* 5.7, 1.6, C_4H), 3.46 (1H, dd, *J* 12.4, 5.7, $C_5H_AH_B$), 3.34 (1H, ddd, *J* 8.3, 6.6, 3.8, C_2H), 2.97 (1H, dd, *J* 13.4, 8.3, CH_XH_YAr), 2.80 (1H, dd, *J* 13.4, 6.6, CH_XH_YAr), 2.71 (1H, dd, *J* 12.4, 2.0 $C_5H_AH_B$); δ_C (62.9 MHz; $CDCl_3$) 157.7 (1C, Q), 131.2 (1C, Q), 129.1 (2C, CH), 112.9 (2C, CH), 76.9 (1C, CH), 76.2 (1C, CH), 62.2 (1C, CH), 53.7 (1C, CH_3), 51.7

(1C, CH_2), 32.7 (1C, CH_2); *m/z* (FAB) 224 ($[M + H]^+$, 100%), 121 (48), 91 (14); HRMS (FAB) (Found: $[M + H]^+$, 224.1285 $C_{12}H_{18}NO_3$ requires *m/z*, 224.1287).

All spectroscopic data was in good agreement with that of the literature.[4]

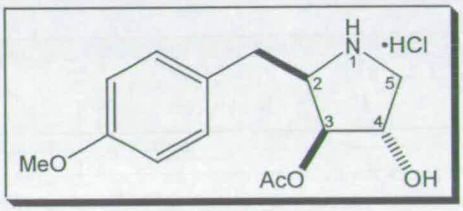
(2*R*,3*S*,4*S*)-3-Acetoxy-4-benzyloxy-1,1-dibenzyl-2-(4-methoxybenzyl)pyrrolidinium chloride 55



To a solution of the chloride salt **48** (0.123 g, 0.232 mmol) in CH_2Cl_2 (5 cm^3) was added DMAP (0.011 g, 0.090 mmol) and freshly distilled acetic anhydride (0.025 g, 0.029 cm^3, 0.30 mmol). The solution was stirred for 18 hours then diluted with water (10 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3). The combined organics were dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [CH_2Cl_2:MeOH (1:19)] to give the ester **55** (0.072 g, 54%) as a foam, R_f [CH_2Cl_2:MeOH (95:5)] 0.10; $[\alpha]_D$ +41.0 (*c* 1.22, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3376, 3031, 2956, 1751, 1612, 1512; δ_H (360 MHz; $CDCl_3$) 7.86–7.09 (15H, m, Ar*H*), 6.96 (2H, d, *J* 8.7, Ar*H*), 6.78 (2H, d, *J* 8.7, Ar*H*), 6.56 (1H, d, *J* 13.6, NCH_AH_BPh), 6.04 (1H, d, *J* 12.5, NCH_XH_YPh), 5.03 (1H, br d, *J* 5.0, C_3H), 4.61 (1H, d, *J* 12.0, OCH_SH_TPh), 4.55 (1H, d, *J* 12.5, NCH_XH_YPh), 4.49 (1H, dd, *J* 13.3, 2.8, CH_MH_NAr), 4.41 (1H, d, *J* 12.0, OCH_SH_TPh), 4.27 (1H, br t, *J* 6.8, C_4H), 4.20 (1H, d, *J* 13.6, NCH_AH_BPh), 4.01 (1H, dd, *J* 13.2, 6.8, $C_5H_EH_F$), 4.00–3.95 (1H, m, C_2H), 3.76 (3H, s, O*Me*), 3.39 (1H, t, *J* 13.3, CH_MH_NAr), 3.22 (1H, dd, *J* 13.2, 6.8, $C_5H_EH_F$), 2.23 (3H, s, $COCH_3$); δ_c (62.9 MHz; $CDCl_3$) 168.4 (1C, Q), 158.7 (1C, Q), 136.0 (1C, Q), 133.8 (2C, CH), 133.4 (2C, CH), 131.1 (1C, CH), 130.8 (1C, CH), 129.5 (2C, CH), 129.4 (4C, CH), 128.6 (2C, CH), 128.4 (1C, CH), 127.8 (2C, CH), 127.2 (1C, Q), 126.6 (1C, Q),

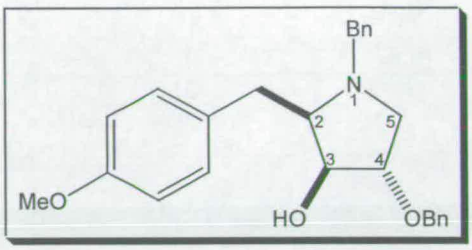
125.9 (1C, Q), 114.4 (2C, CH), 78.8 (1C, CH), 74.3 (1C, CH), 72.6 (1C, CH), 72.4 (1C, CH_2), 62.4 (1C, CH_2), 62.2 (1C, CH_2), 58.6 (1C, CH_2), 55.1 (1C, CH_3), 27.5 (1C, CH_2), 20.9 (1C, CH_3); *m/z* (FAB) 536 ($[M + H]^+$, 69%), 446 (35), 174 (15), 121 (39), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 536.2808. $[C_{35}H_{38}NO_4]^+Cl^-$ requires *m/z,* 536.2801).

(2*R*,3*S*,4*S*)-3-Acetoxy-4-hydroxy-2-(4-methoxybenzyl)pyrrolidine hydrochloride 23



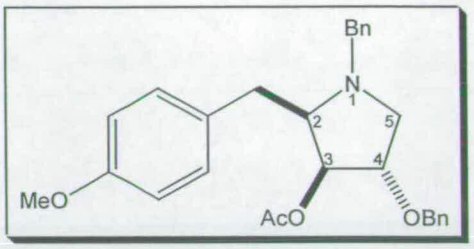
A solution of the chloride salt **55** (0.070 g, 0.12 mmol) and Pearlman's catalyst [0.070 g; 20% $Pd(OH)_2/C$] in methanol (5 cm^3) was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 24 hours. The mixture was then filtered through a pad of Cēlite and concentrated under reduced pressure to give anisomycin as its hydrochloride salt **23** (0.036 g, 100%), mp 187–188 °C; $[\alpha]_D$ +4.0 (*c* 0.28, CH_3OH) [lit.,[142] 187– 188 °C, $[\alpha]_D$ +4.2 (*c* 0.51, CH_3OH)]; ν_{max} (KBr)/cm^{-1} 3395, 3291, 3252, 1749, 1612, 1514; δ_H (250 MHz; CD_3OD) 7.26 (2H, d, *J* 8.6, Ar*H*), 6.95 (2H, d, *J* 8.6, Ar*H*), 5.08 (1H, d, *J* 3.0, C_3H), 4.38 (1H, d, *J* 4.3, C_4H), 4.23-4.15 (1H, m, C_2H), 3.81 (3H, s, O*Me*), 3.63 (1H, dd, *J* 12.8, 4.3, $C_5H_AH_B$), 3.22 (1H, d, *J* 12.8, $C_5H_AH_B$), 3.11 (1H, dd, *J* 14.3, 6.7, CH_XH_YAr), 2.99 (1H, dd, *J* 14.3, 8.8, CH_XH_YAr), 2.21 (3H, s, $COCH_3$); δ_c (62.9 MHz; CD_3OD) 169.8 (1C, Q), 159.6 (1C, Q), 130.0 (2C, CH), 127.9 (1C, Q), 114.5 (2C, CH), 77.3 (1C, CH), 72.4 (1C, CH), 62.7 (1C, CH), 54.7 (1C, CH_3), 51.6 (1C, CH_2), 31.2 (1C, CH_2), 19.7 (1C, CH_3); *m/z* (FAB) 266 ($[M + H]^+$, 77%), 206 (55), 149 (22), 134 (59), 121 (26), 91 (41); HRMS (FAB) (Found: $[M + H]^+$, 266.1385. $[C_{14}H_{20}NO_4]^+Cl^-$ requires *m/z* 266.1392).

(2*R*,3*S*,4*S*)-1-Benzyl-4-benzyloxy-3-hydroxy-2-(4-methoxybenzyl)pyrrolidine 56



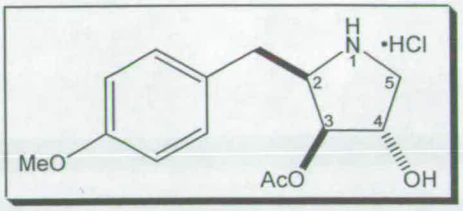
To a solution of the chloride salt **48** (0.049 g, 0.093 mmol) in methanol (5 cm^3) was added 5% Pd/C (0.0049 g) and potassium carbonate (0.045 g, 0.32 mmol). The mixture was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 10 minutes. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. To the residue was added CH_2Cl_2 (25 cm^3) and water (25 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3); the combined organic phase was then washed with saturated aq. sodium bicarbonate (25 cm^3) then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane-EtOAc (7:3)] to give pyrrolidine **56** (0.035 g, 94%) as an oil, R_f [hexane: EtOAc (1:1)] 0.33; $[\alpha]_D$ -96.1 (*c* 0.4, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3419, 2917, 1610, 1511; δ_H (250 MHz; $CDCl_3$) 7.33-7.19 (12H, m, Ar*H*), 6.84 (2H, d, *J* 8.7, Ar*H*), 4.46 (1H, d, *J* 11.9, OC$H_A$$H_B$Ph), 4.39 (1H, d, *J* 11.9, OC$H_A$$H_B$Ph), 4.14 (1H, d, *J* 12.9, NC$H_X$$H_Y$Ph), 3.82–3.77 (1H, m, C_3H), 3.80 (1H, dd, *J* 6.6, 4.1, C_4H), 3.79 (3H, s, O*Me*), 3.33 (1H, d, *J* 12.9, NC$H_X$$H_Y$Ph), 3.32 (1H, dd, *J* 10.5, 6.6, $C_5H_S$$H_T$), 2.97–2.81 (3H, m, C$H_M$$H_N$Ar, + C$H_M$$H_N$Ar + C_2H), 2.20 (1H, dd, *J* 10.5, 4.4, $C_5H_S$$H_T$); δ_c (62.9 MHz; $CDCl_3$) 157.9 (1C, Q), 138.4 (1C, Q), 137.8 (1C, Q), 130.8 (1C, Q), 130.1 (2C, CH), 128.7 (2C, CH), 128.3 (2C, CH), 128.2 (2C, CH), 127.5 (3C, CH), 127.0 (1C, CH), 113.8 (2C, CH), 82.6 (1C, CH), 75.4 (1C, CH), 71.2 (1C, CH_2), 68.2 (1C, CH), 58.4 (1C, CH_2), 57.7 (1C, CH_2), 55.1 (1C, CH_3), 32.5 (1C, CH_2); *m/z* (FAB) 404 ($[M + H]^+$, 16%), 307 (19), 154 (100), 136 (72), 91 (30); HRMS (FAB) (Found: $[M + H]^+$, 404.2226. $C_{26}H_{30}NO_3$ requires *m/z* 404.2226).

(2*R*,3*S*,4*S*)-3-Acetoxy-1-benzyl-4-benzyloxy-2-(4-methoxybenzyl)pyrrolidine 57



To a solution of the alcohol **56** (0.026 g, 0.065 mmol) in CH_2Cl_2 (4 cm^3) was added freshly distilled acetic anhydride (0.013 g, 0.015 cm^3, 0.13 mmol), and triethylamine (0.013 g, 0.018 cm^3, 0.13 mmol). The solution was stirred for 18 hours and then quenched by the addition of saturated aq. sodium bicarbonate (25 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 cm^3); the combined organic phase was dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give the ester **57** (0.026 g, 92%) as a colourless oil, R_f [hexane: EtOAc (7:3)] 0.49; $[\alpha]_D$ -105.7 (*c* 1.1, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2932, 1738, 1612, 1583, 1512; δ_H (250 MHz; $CDCl_3$) 7.33–7.20 (10H, m, Ar*H*), 7.07 (2H, d, *J* 8.8, Ar*H*), 6.81 (2H, d, *J* 8.8, Ar*H*), 4.91 (1H, dd, *J* 5.0, 1.5, C_3H), 4.62 (1H, d, *J* 12.1, OCH_AH_BPh), 4.45 (1H, d, *J* 12.1, OCH_AH_BPh), 4.05 (1H, d, *J* 13.1, NCH_XH_YPh), 3.82–3.71 (1H, m, C_4H), 3.77 (3H, s, O*Me*), 3.38 (1H, d, *J* 13.1, NCH_XH_YPh), 3.33 (1H, dd, *J* 10.7, 6.7, $C_5H_SH_T$), 3.10 (1H, dt, *J* 9.6, 5.0, C_2H), 2.96 (1H, dd, *J* 13.6, 5.0, CH_MH_NAr), 2.76 (1H, dd, *J* 13.6, 9.6, CH_MH_NAr), 2.32 (1H, dd, *J* 10.7, 4.9, $C_5H_SH_T$), 2.11 (3H, s, $COCH_3$); δ_c (62.9 MHz; $CDCl_3$) 170.2 (1C, Q), 157.9 (1C, Q), 138.2 (1C, Q), 137.9 (1C, Q), 130.6 (1C, Q), 129.6 (3C, CH), 128.9 (2C, CH), 128.2 (3C, CH), 127.5 (2C, CH), 127.4 (1C, CH), 127.0 (1C, CH), 113.8 (2C, CH), 81.0 (1C, CH), 77.8 (1C, CH), 71.3 (1C, CH_2), 66.5 (1C, CH), 58.3 (2C, CH_2), 55.1 (1C, CH_3), 33.1 (1C, CH_2), 21.1 (1C, CH_3); *m/z* (FAB) 446 ($[M + H]^+$, 35%), 356 (10), 324 (10), 217 (19), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 446.2332. $C_{28}H_{32}NO_4$ requires *m/z* 446.2331).

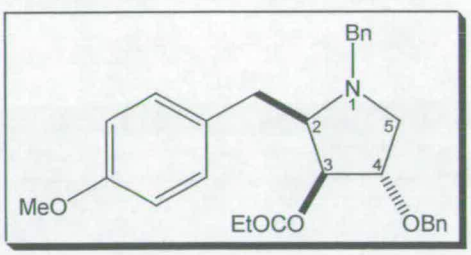
(2*R*,3*S*,4*S*)-3-Acetoxy-4-hydroxy-2-(4-methoxybenzyl)pyrrolidine hydrochloride 23



To a solution of the pyrrolidine **57** (0.019 g, 0.043 mmol) and Pearlman's catalyst [0.019 g; 20% $Pd(OH)_2/C$] in methanol (3 cm^3) was added hydrochloric acid (0.09 cm^3, 1 M in ether, 0.09 mmol). The solution was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 1 hour. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give anisomycin as it's hydrochloride salt **23** (0.013 g, 100%) as a solid.

All spectroscopic data was identical to that obtained previously.

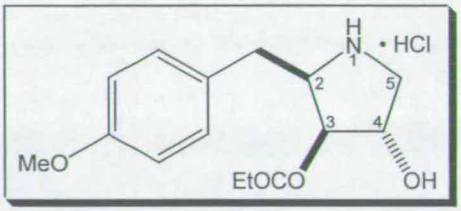
(2*R*,3*S*,4*S*)-3-Propyloxy-1-benzyl-4-benzyloxy-2-(4-methoxybenzyl)pyrrolidine 58



To a solution of the alcohol **56** (0.0803 g, 0.258 mmol) in CH_2Cl_2 (6 cm^3) was added freshly distilled propanoic anhydride (0.067 g, 0.066 cm^3, 0.52 mmol), and triethylamine (0.052 g, 0.072 cm^3, 0.52 mmol). The solution was stirred for 18 hours and then quenched by the addition of saturated aq. sodium bicarbonate (15 cm^3). The

organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 15 cm^3); the combined organic phase was dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane-EtOAc (4:1)] to give the ester **58** (0.0848g, 89%) as a colourless oil, R_f [hexane: EtOAc (7:3)] 0.49; $[\alpha]_D$ –80.0 (*c* 0.58, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3061, 2937, 1735, 1612, 1583, 1512; δ_H (360 MHz; $CDCl_3$) 7.44–7.31 (10H, m, Ar*H*), 7.17 (2H, d, *J* 8.7, Ar*H*), 6.91 (2H, d, *J* 8.7, Ar*H*), 5.12 (1H, dd, *J* 5.1, 1.5, C_3H), 4.73 (1H, d, *J* 12.1, OCH_AH_BPh), 4.57 (1H, d, *J* 12.1 OCH_AH_BPh), 4.14 (1H, d, *J* 13.1, NCH_XH_YPh), 3.91 (1H, ddd, *J* 6.7, 4.9, 1.5, C_4H), 3.87 (3H, s, O*Me*), 3.47 (1H, d, *J* 13.1, NCH_XH_YPh), 3.43 (1H, dd, *J* 10.8, 6.7, $C_5H_SH_T$), 3.23 (1H, dt, *J* 9.4, 5.1, C_2H), 3.04 (1H, dd, *J* 13.8, 5.1, CH_MH_NAr), 2.88 (1H, dd, *J* 13.8, 9.4, CH_MH_NAr), 2.50 (2H, q, *J* 7.6, CH_2CH_3), 2.44 (1H, dd, *J* 10.8, 4.9, $C_5H_SH_T$), 1.27 (3H, t, 7.6, CH_2CH_3); δ_c (62.9 MHz; $CDCl_3$) 173.6 (1C, Q), 157.9 (1C, Q), 138.3 (1C, Q), 138.0 (1C, Q), 130.6 (1C, Q). 129.6 (2C, CH), 128.8 (2C, CH), 128.1 (2C, CH), 128.0 (2C, CH), 127.5 (2C, CH), 127.4 (1C, CH), 126.9 (1C, CH), 113.7 (2C, CH), 81.1 (1C, CH), 77.5 (1C, CH), 71.3 (1C, CH_2), 66.5 (1C, CH), 58.4 (1C, CH_2), 58.3 (1C, CH_2), 55.0 (1C, CH_3), 33.1 (1C, CH_2), 27.6 (1C, CH_2), 9.0 (1C, CH_3); *m/z* (FAB) 460 ($[M + H]^+$, 31%), 458 (46), 338 (53), 174 (54) 121 (75), 91(100); HRMS (FAB) (Found: $[M + H]^+$, 460.2494. $C_{29}H_{34}NO_4$ requires *m/z* 460.2488).

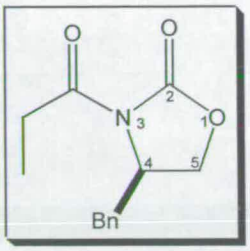
(2*R*,3*S*,4*S*)-3-Propyloxy-4-hydroxy-2-(4-methoxybenzyl)pyrrolidine hydrochloride 59



To a solution of the pyrrolidine **58** (0.028 g, 0.062 mmol) and Pearlman's catalyst [0.028 g; 20% $Pd(OH)_2/C$] in methanol (3 cm^3) was added hydrochloric acid (0.12

cm^3, 1 M in ether, 0.12 mmol). The solution was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 18 hours. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give 3097-B1 as it's hydrochloride salt **59** (0.020 g, 100%) as a colourless solid, mp 213–215 °C; $[\alpha]_D$ +6.3 (*c* 0.16, CH_3OH); ν_{max} (KBr)/cm^{-1} 3346, 3251, 2876, 1730, 1612, 1581, 1514; δ_H (250 MHz; CD_3OD) 7.32 (2H, d, *J* 8.7, Ar*H*), 7.01 (2H, d, *J* 8.7, Ar*H*), 5.17 (1H, d, *J* 2.9, C_3H), 4.43 (1H, d, *J* 3.9, C_4H), 4.30–4.23 (1H, m, C_2H), 3.87 (3H, s, O*Me*), 3.69 (1H, dd, *J* 12.8, 3.9, $C_5H_AH_B$), 3.29 (1H, d, *J* 12.8, $C_5H_AH_B$), 3.17 (1H, dd, *J* 14.3, 6.9, CH_XH_YAr), 3.05 (1H, dd, *J* 14.3, 8.7, CH_XH_YAr), 2.60 (2H, m, CH_2CH_3), 1.28 (3H, t, *J* 7.5, CH_2CH_3); δ_c (62.9 MHz; CD_3OD) 172.3 (1C, Q), 158.7 (1C, Q), 129.0 (2C, CH), 127.0 (1C, Q), 113.6 (2C, CH), 76.2 (1C, CH), 71.5 (1C, CH), 61.8 (1C, CH), 53.8 (1C, CH_3), 50.7 (1C, CH_2), 30.3 (1C, CH_2), 26.2 (1C, CH_2), 7.4 (1C, CH_3); *m/z* (FAB) 280 ($[M + H]^+$, 100%), 136 (94), 154 (95), 107 (73), 121 (76), 91 (69); HRMS (FAB) (Found: $[M + H]^+$, 280.1543. $[C_{15}H_{22}NO_4]Cl^-$ requires *m/z* 280.1549).

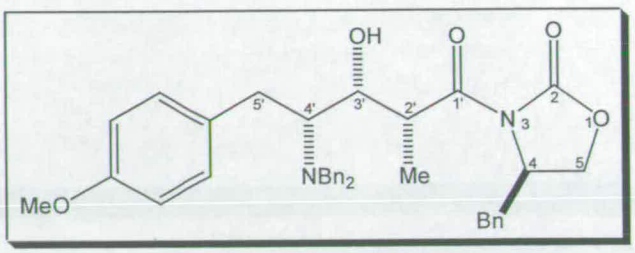
(4*R*)-3-(1'-Oxopropyl)-4-phenylmethyloxazolidin-2-one 91



To a solution of oxazolidinone **45** (2.50 g, 14.1 mmol) in THF (50 cm^3) at -78 °C was added *n*-butyllithium (8.45 cm^3, 1.67 M in hexanes, 14.1 mmol) and the solution turned orange. The solution was allowed to stir for 15 minutes before freshly distilled propionyl chloride (4.17 cm^3, 3.91 g, 42.3 mmol) was added causing the orange colour to disappear. The mixture was left to stir for 2 hours and was then quenched by the addition of a saturated aqueous solution of ammonium chloride (50 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 30 cm^3); the combined organic phase was washed sequentially with saturated aq. sodium bicarbonate (100 cm^3) and brine (100 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (4:1)] to give **91** (2.96 g, 90%) as an oil, R_f [hexane:EtOAc (7:3)] 0.48; $[\alpha]_D$ -95.1 (*c* 1.0, EtOH) [lit.(*ent*-**91**),[141] $[\alpha]_D$ +92.9 (*c* 1.01, EtOH)]; ν_{max} (neat)/cm^{-1} 2980, 1781, 1703, 1604, 1497; δ_H (250 MHz; $CDCl_3$) 7.54–7.35 (5H, m, Ar*H*), 4.11 (1H, dddd, *J* 9.8, 9.3, 6.8, 3.4 C_4H), 4.37–4.33 (1H, m, $C_5H_AH_B$), 4.34 (1H, dd, *J* 9.9, 9.3, $C_5H_AH_B$), 3.46 (1H, dd, *J* 13.3, 3.4, CH_XH_YPh), 3.12 (2H, q, *J* 7.4, CH_2CH_3), 2.93 (1H, dd, 13.3, 9.8, CH_XH_YPh), 1.37 (3H, t, *J* 7.4, CH_2CH_3); δ_c (62.9 MHz; $CDCl_3$) 174.2 (1C, Q), 153.6 (1C, Q), 135.4 (1C, Q), 129.5 (2C, CH), 129.0 (2C, CH), 127.4 (1C, CH), 66.3 (1C, CH_2), 55.3 (1C, CH), 38.0 (1C, CH_2), 29.3 (1C, CH_2), 8.4 (1C, CH_3); *m/z* (FAB) 234 ($[M + H]^+$, 96%), 218 (48), 178 (89), 117 (76), 91 (78); HRMS (FAB) (Found: $[M + H]^+$, 234.1136. $C_{13}H_{16}NO_3$ requires *m/z* 234.1130).

All spectroscopic data was in good agreement with that of the literature.[141]

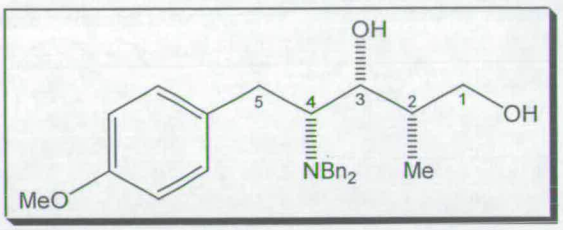
(2'*R*,3'*R*,4*R*,4'*R*)-3-(4'-*N*,*N*-dibenzylamino-3'-hydroxy-5'-(4-methoxyphenyl)-2'-methyl-1'-oxopentyl)-4-phenylmethyloxazolidin-2-one 90



To a solution of propionate equivalent **91** (0.202 g, 0.858 mmol) in CH_2Cl_2 (3 cm^3) at 0 °C was added, dropwise, dibutylboron triflate (0.92 cm^3, 1.0 M in CH_2Cl_2, 0.92 mmol) followed by triethylamine (0.10 g, 0.13 cm^3, 0.95 mmol). The solution was stirred at 0 °C for 15 min, then cooled to -78 °C and a -78 °C solution of aldehyde **31** (0.200 g, 0.557 mmol) in CH_2Cl_2 (3 cm^3) was added dropwise *via* cannula. The reaction mixture was stirred at -78 °C for 1 hour, and allowed to warm to 0 °C over a period of 4 h. The reaction was quenched at 0 °C by the addition of methanol (5 cm^3) followed by pH 7 phosphate buffer (1 cm^3). Hydrogen peroxide (30% aq. solution; 5 cm^3 in methanol (5 cm^3) was added dropwise to the solution and the mixture was stirred and allowed to warm to room temperature over *ca.* 1 hour. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was washed sequentially with saturated aq. sodium bicarbonate (25 cm^3) and brine (25 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed using preparative HPLC [hexane:EtOAc (7:3)] to give **90** (0.144 g, 87%) as an oil, R_f [hexane:EtOAc (7:3)] 0.40; R_t [hexane:EtOAc (7:3)] = 11.08 minutes; $[\alpha]_D$ -48.7 (*c* 2.00, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3370, 2929, 1773, 1703, 1612, 1511; δ_H (200 MHz; $CDCl_3$) 7.36–6.90 (17H, m, Ar*H*), 6.87 (2H, d, *J* 8.7, Ar*H*), 4.65–4.58 (2H, m, C_4H + O*H*), 4.22–4.09 (2H, m, C_5H_2), 3.91 (1H, dd, *J* 9.0, 2.5, C_3·*H*), 3.87 (2H, d, *J* 12.8, NCH_AH_BPh × 2), 3.81 (3H, s, O*Me*), 3.72 (1H, qd, *J* 6.9, 2.5, C_2·*H*), 3.40 (2H, d, *J* 12.8, NCH_AH_BPh × 2), 3.33 (1H, dd, *J* 13.3, 3.0, $C_4HCH_MH_NPh$), 3.13–2.97 (2H, m, C_4·*H* + C_5·H_XH_Y), 2.85–2.76 (1H, m, C_5·H_XH_Y), 2.67 (1H, dd, *J* 13.3, 10.0, $C_4HCH_MH_NPh$), 0.61 (3H, d, *J*

6.9, C_2·H*Me*); δ_C (62.9 MHz, $CDCl_3$) 175.3 (1C, Q), 158.5 (1C, Q), 153.8 (1C, Q), 139.0 (2C, Q), 136.0 (1C, Q), 132.3 (1C, Q), 130.7 (2C, CH)), 129.9 (2C, CH), 129.5 (4C, CH), 129.3 (2C, CH), 129.0 (4C, CH), 127.8 (2C, CH), 127.7 (1C, CH), 114.4 (2C, CH), 70.5 (1C, CH), 66.6 (1C, CH_2), 61.6 (1C, CH), 56.4 (1C, CH_3), 55.7 (1C, CH), 54.3 (2C, CH_2), 40.6 (1C, CH), 38.1 (1C, CH_2), 31.2 (1C, CH_2), 8.7 (1C, CH_3); *m/z* (FAB) 593 ($[M + H]^+$, 59%), 307 (16), 154 (100), 136 (70), 121 (16), 91 (51); HRMS (FAB) (Found: $[M + H]^+$, 593.3018. $C_{37}H_{41}N_2O_5$ requires *m/z*, 593.3015).

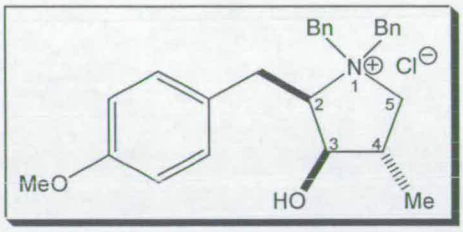
(2*S*,3*R*,4*R*)-4-*N*,*N*,-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)-2-methylpentan-1-ol 89



To a solution of aldol adduct **90** (0.281 g, 0.475 mmol) in THF (20 cm^3) at 0 °C was added methanol (0.076 g, 0.096 cm^3, 2.4 mmol) and $LiBH_4$ (0.052 g, 1.1 mmol). The solution was warmed to room temperature and stirred for 18 hours then re-cooled to 0 °C and quenched by the addition of 1 M aqueous sodium hydroxide (2 cm^3). The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was washed with brine (30 cm^3), dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give the diol **89** (0.162 g, 82%) as an oil, R_f [hexane:EtOAc (7:3)] 0.12; $[\alpha]_D$ -31.72 (*c* 1.95, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3397, 3027, 2933, 1611, 1583, 1512; δ_H (250 MHz; $CDCl_3$) 7.34–7.10 (10H, m, Ar*H*), 7.13 (2H, d, *J* 8.7, Ar*H*), 6.88 (2H, d, *J* 8.7, Ar*H*), 4.74 (1H, s, CHO*H*), 3.90–3.78 (1H, m, C_3H), 3.88 (2H, d, *J* 12.7, NCH_AH_BPh × 2), 3.82 (3H, s, O*Me*), 3.68 (1H, dd, *J* 10.6, 4.2, CH_MH_NOH), 3.58 (1H, dd, *J* 10.6, 6.0, CH_MH_NOH), 3.36 (2H, d, *J* 12.7, NCH_AH_BPh

× 2), 3.07–2.97 (1H, m, C_4H), 3.02 (1H, dd, *J* 17.2, 9.1, $C_5H_XH_Y$), 2.67 (1H, s, CH_2OH), 2.55 (1H, dd, *J* 17.2, 8.4, $C_5H_XH_Y$), 1.52 (1H, m, C_2H), 0.39 (3H, d, *J* 6.8, CH*Me*); δ_C (62.9 MHz, $CDCl_3$) 158.0 (1C, Q), 138.4 (2C, Q), 131.7 (1C, Q), 130.0 (2C, CH), 129.0 (4C, CH), 128.3 (4C, CH), 127.2 (2C, CH), 113.9 (2C, CH), 71.6 (1C, CH), 67.6 (1C, CH_2), 60.3 (1C, CH), 55.1 (1C, CH_3), 53.5 (2C, CH_2), 35.8 (1C, CH), 31.7 (1C, CH_2) , 8.2 (1C, CH_3); *m/z* (FAB) 420 ($[M + H]^+$, 67%), 330 (66), 298 (62), 240 (21), 208 (15), 121 (75), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 420.2538. $C_{27}H_{34}NO_3$ requires *m/z,* 420.2539).

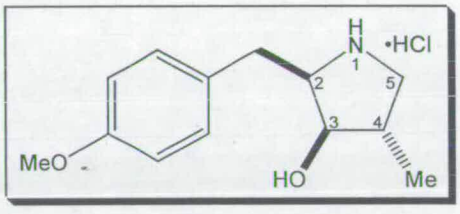
(2*R*,3*R*,4*S*)-1,1-dibenzyl-3-hydroxy-2-(4-methoxybenzyl)-4-methylpyrrolidinium chloride 92



To a solution of the diol **89** (0.125 g, 0.297 mmol) in CH_2Cl_2 (10 cm^3) at 0 °C was added DMAP (0.163 g, 1.34 mmol) and TsCl (0.170 g, 0.892 mmol). The solution was stirred for 18 hours then diluted with CH_2Cl_2 (25 cm^3) and water (25 cm^3). The organic phase was separated and washed with 1% aqueous hydrochloric acid (2 × 25 cm^3) then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [CH_2Cl_2:MeOH (100:0) → (95:5)] to give a white foam. The salt obtained was subjected to ion exchange chromatography [Dowex Cl^-; prepared by treatment of Dowex 1-X2 with 1% aqueous hydrochloric acid followed by flushing with methanol until the eluent returned to pH 7)] eluting with methanol to give the chloride salt **92** (0.108 g, 83%) as an amorphous solid, R_f [CH_2Cl_2:MeOH (95:5)] 0.05; $[\alpha]_D$ -41.3 (*c* 3.08, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3182, 2960, 1612, 1513; δ_H (360 MHz; $CDCl_3$) 7.76 (2H, d, *J* 7.0, Ar*H*), 7.44–7.30 (10H, m, Ar*H*), 6.83 (1H, d, *J* 3.6, C_3HOH), 6.69 (2H, d, *J* 8.6, Ar*H*), 5.78 (1H, d, *J* 13.4, NCH_AH_BPh), 5.24 (1H,

d, *J* 13.0, $NCH_X H_Y Ph$), 5.09 (1H, d, *J* 13.0, $NCH_X H_Y Ph$), 4.14 (1H, d, *J* 13.4 $NCH_A H_B Ph$), 3.95–3.92 (2H, m, C_3H + $CH_A H_B Ar$), 3.69 (3H, s, O*Me*), 3.66–3.56 (2H, m, $CH_A H_B Ar$ + $C_5H_A H_B$), 3.31–3.28 (1H, m, C_2H), 3.26–3.04 (1H, m, C_4H), 2.56 (1H, br t, *J* 11.1, $C_5H_A H_B$), 0.91 (3H, d, *J* 6.8, C_4HMe); δ_C (62.9 MHz; $CDCl_3$) 158.2 (1C, Q), 133.4 (2C, CH), 133.2 (2C, CH), 130.5 (3C, CH), 130.2 (1C, CH), 129.1 (2C, CH), 129.0 (2C, CH), 127.8 (1C, Q), 127.4 (1C, Q), 126.8 (1C, Q), 113.9 (2C, CH), 74.4 (1C, CH), 74.3 (1C, CH), 61.2 (1C, CH_2), 60.6 (1C, CH_2), 59.9 (1C, CH_2), 55.0 (1C, CH_3), 38.7 (1C, CH), 26.8 (1C, CH_2), 17.0 (1C, CH_3); *m/z* (FAB) 402 ($[M]^+$, 66%), 312 (65), 190 (63), 121 (76), 91 (100); HRMS (FAB) (Found: $[M]^+$, 402.2433. $[C_{27}H_{32}NO_2]^+Cl^-$ requires *m/z*, 402.2433).

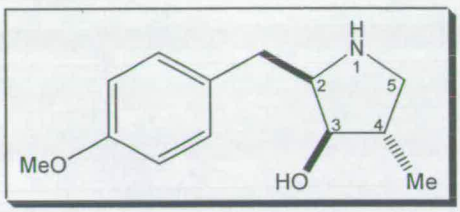
(2*R*,3*R*,4*S*)-3-hydroxy-2-(4-methoxybenzyl)-4-methylpyrrolidine hydrochloride 95



A solution of the chloride salt **92** (0.044 g, 0.10 mmol) and Pearlman's catalyst [0.044 g; 20% $Pd(OH)_2/C$] in methanol (5 cm^3) was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 24 hours. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give **95** (0.026 g, 100%) as a white solid, mp 191–192 °C; $[\alpha]_D$ +33.3 (*c* 0.27, CH_3OH); ν_{max} (KBr)/cm^{-1} 3356, 2956, 2920, 1614, 1577, 1516; δ_H (250 MHz; CD_3OD) 7.40 (2H, d, *J* 8.1, Ar*H*), 7.03 (2H, d, *J* 8.1, Ar*H*), 4.05 (1H, br s, C_3H), 3.90 (3H, s, O*Me*), 3.84–3.83 (1H, m, C_2H), 3.74 (1H, dd, *J* 11.4, 7.5, $C_5H_A H_B$), 3.27 (1H, dd, *J* 14.2, 6.1, $CH_X H_Y Ar$), 3.05 (1H, dd, *J* 14.2, 8.6, $CH_X H_Y Ar$), 2.97 (1H, dd, *J* 11.4, 3.2, $C_5H_A H_B$), 2.24–2.12 (1H, m, C_4H), 1.19 (3H, d, *J* 7.3, C_4HMe); δ_c (62.9 MHz; CD_3OD) 157.9 (1C, Q), 128.7 (2C, CH), 127.4 (1C, Q), 112.9 (2C, CH), 74.5 (1C, CH), 63.1 (1C, CH), 53.2 (1C, CH_3), 48.6 (1C, CH_2), 39.7 (1C, CH), 30.1 (1C, CH_2),

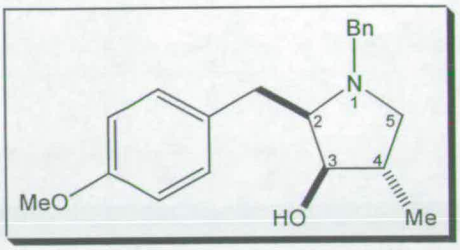
15.1 (1C, CH_3); *m/z* (FAB) 222 ($[M + H]^+$, 68%), 206 (47), 134 (52), 121 (40), 91 (68); HRMS (FAB) (Found: $[M + H]^+$, 222.1494. $[C_{13}H_{20}NO_2]^+Cl^-$ requires *m/z* 222.1494).

(2*R*,3*R*,4*S*)-3-hydroxy-2-(4-methoxybenzyl)-4-methylpyrrolidine 83



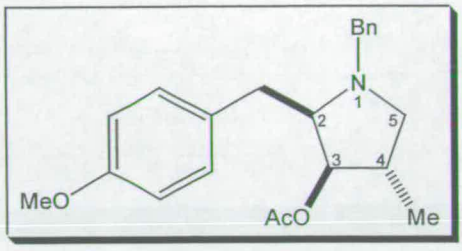
The HCl salt **95** was subjected to ion-exchange chromatography [Dowex OH^-] (prepared by treating Dowex 1-X2 with 1 M aqueous NaOH, followed by methanol until the pH of the eluent returned to 7). Eluting with methanol gave the free base (quantitative recovery) as a white solid, R_f [CH_2Cl_2:MeOH (95:5)] 0.04; mp 108–110 °C; $[\alpha]_D$ +5.7 (*c* 0.35, CH_3OH); ν_{max} (KBr)/cm^{-1} 3420, 3260, 2951, 1612, 1584, 1512; δ_H (250 MHz; CD_3OD) 7.28 (2H, d, *J* 8.7, Ar*H*), 6.92 (2H, d, *J* 8.7, Ar*H*), 3.84 (3H, s, O*Me*), 3.71 (1H, dd, *J* 2.4, 1.7, C_3H), 3.37 (1H, dd, *J* 11.1, 7.8, $C_5H_AH_B$), 3.19–3.11 (1H, m, C_2H) 2.99 (1H, dd, *J* 13.6, 6.8, CH_MH_NAr), 2.74 (1H, dd, *J* 13.6, 7.6, CH_MH_NAr), 2.38 (1H, dd, *J* 11.1, 5.7, $C_5H_AH_B$), 2.24–2.15 (1H, m, C_4H), 1.07 (3H, d, *J* 7.2, C_4H*Me*); δ_c (62.9 MHz; CD_3OD) 157.7 (1C, Q), 131.2 (1C, Q), 129.1 (2C, CH), 112.9 (2C, CH), 77.9 (1C, CH), 63.1 (1C, CH), 53.7 (1C, CH_3), 50.9 (1C, CH_2), 41.7 (1C, CH), 33.3 (1C, CH_2), 16.5 (1C, CH_3); *m/z* (FAB) 222 ($[M + H]^+$, 100%), 121 (17), 91 (95), 57 (66); HRMS (FAB) (Found: $[M + H]^+$, 222.1495. $C_{13}H_{20}NO_2$ requires *m/z* 222.1494).

(2*R*,3*R*,4*S*)-1-Benzyl-3-hydroxy-2-(4-methoxybenzyl)-4-methylpyrrolidine 88



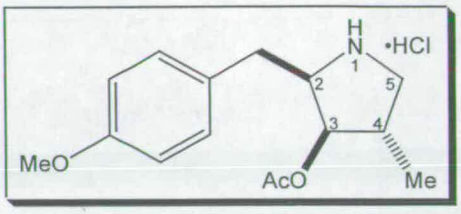
To a solution of the chloride salt **92** (0.0782 g, 0.178 mmol) in methanol (5 cm^3) was added 5% Pd/C (0.0078 g) and potassium carbonate (0.074 g, 0.54 mmol). The mixture was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 20 minutes. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. To the residue was added CH_2Cl_2 (25 cm^3) and water (25 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was washed with saturated aq. sodium bicarbonate (50 cm^3) then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give pyrrolidine **88** (0.0518 g, 93%) as an oil, R_f [hexane:EtOAc (1:1)] 0.33; $[\alpha]_D$ -78.3 (*c* 1.5, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3419, 2954, 1611, 1583, 1512, 1246; δ_H (250 MHz; $CDCl_3$) 7.33–7.23 (5H, m, Ar*H*), 7.26 (2H, d, *J* 8.7, Ar*H*), 6.85 (2H, d, *J* 8.7, Ar*H*), 4.10 (1H, d, *J* 12.7, NC$H_A$$H_B$Ph), 3.78 (3H, s, O*Me*), 3.50 (1H, dd, *J* 4.7, 2.6, C_3H), 3.16 (1H, d, *J* 12.7, NC$H_A$$H_B$Ph), 3.13 (1H, dd, *J* 9.4, 7.9, $C_5H_SH_T$), 2.94 (1H, dd, *J* 13.4, 8.9, C$H_X$$H_Y$Ar), 2.87 (1H, dd, *J* 13.4, 5.3, C$H_X$$H_Y$Ar), 2.57 (1H, dt, *J* 8.9, 5.0, C_2H), 2.35 (1H, br s, O*H*), 2.03–1.94 (1H, m, C_4H), 1.67 (1H, dd, *J* 9.4, 7.9, $C_5H_SH_T$), 0.91 (3H, d, *J* 7.1, CH*Me*); δ_c (62.9 MHz; $CDCl_3$) 157.8 (1C, Q), 138.6 (1C, Q), 131.4 (1C, Q), 130.1 (2C, CH), 128.8 (2C, CH), 128.1 (2C, CH), 126.9 (1C, CH), 113.7 (2C, CH), 78.8 (1C, CH), 69.3 (1C, CH), 60.2 (1C, CH_2), 58.2 (1C, CH_2), 55.1 (1C, CH_3), 40.1 (1C, CH), 32.9 (1C, CH_2), 18.0 (1C, CH_3); *m/z* (FAB) 312 ($[M + H]^+$, 73%), 190 (65), 121 (41), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 312.1963. $C_{20}H_{26}NO_2$ requires *m/z* 312.1964).

(2*R*,3*R*,4*S*)-3-Acetoxy-1-benzyl-2-(4-methoxybenzyl)-4-methylpyrrolidine 93



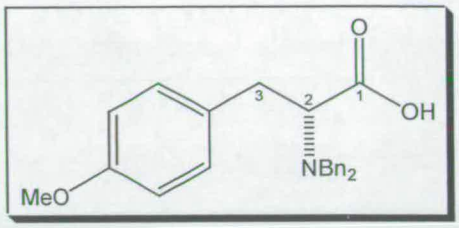
To a solution of the alcohol **88** (0.0322 g, 0.104 mmol) in CH_2Cl_2 (5 cm^3) was added freshly distilled acetic anhydride (0.021 g, 0.020 cm^3, 0.21 mmol), and triethylamine (0.021 g, 0.030 cm^3, 0.21 mmol). The solution was stirred for 18 hours before being diluted with CH_2Cl_2 (10 cm^3) and saturated aq. sodium bicarbonate (20 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 cm^3); the combined organic phase was dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give the ester **93** (0.0300g, 82%) as a colourless oil, R_f [hexane:EtOAc (7:3)] 0.31; $[\alpha]_D$ -73.96 (*c* 0.70, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2959, 2789, 1734, 1612, 1512 cm^{-1}; δ_H (250Hz; $CDCl_3$), 7.31–7.22 (5H, m, Ar*H*), 7.10 (2H, d, *J* 8.6, Ar*H*), 6.80 (2H, d, *J* 8.6, Ar*H*), 4.62–4.58 (1H, m, C_3H), 3.96 (1H, d, *J* 12.9, NCH_XH_YPh), 3.77 (3H, s, O*Me*), 3.24 (1H, d, *J* 12.9, NCH_XH_YPh), 3.12 (1H, dd, *J* 9.3, 7.5, $C_5H_MH_N$), 2.94–2.80 (2H, m, $CH_AH_BAr + C_2H$), 2.83 (1H, dd, *J* 14.2, 8.9, CH_AH_BAr), 2.11–2.03 (1H, m, C_4H), 2.06 (3H, s, $COCH_3$), 1.80 (1H, t, *J* 9.3, $C_5H_MH_N$), 0.99 (3H, d, *J* 7.0, C_4HMe); δ_c (62.9 Hz; $CDCl_3$) 170.8 (1C, Q), 157.9 (1C, Q), 138.3 (1C, Q), 131.1 (1C, Q), 129.8 (2C, CH), 129.1(2C, CH), 128.2 (2C, CH), 127.0 (1C, CH), 113.8 (2C, CH), 80.9 (1C, CH), 67.1 (1C, CH), 60.0 (1C, CH_2), 58.8 (1C, CH_2), 55.2 (1C, CH_3), 38.3 (1C, CH), 33.8 (1C, CH_2), 21.2 (1C, CH_3), 17.1 (1C, CH_3); *m/z* (FAB) 354 ($[M + H]^+$, 39%), 352 (52), 294 (34), 232 (63), 172 (28), 121 (75), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 354.2069. $C_{22}H_{28}NO_3$ requires *m/z* 354.2069).

(2*R*,3*R*,4*S*)-3-Acetoxy-2-(4-methoxybenzyl)-4-methylpyrrolidine hydrochloride 94



To a solution of the pyrrolidine **93** (0.0407 g, 0.115 mmol) and Pearlman's catalyst [0.0407 g; 20% $Pd(OH)_2/C$] in methanol (5 cm^3) was added hydrochloric acid (0.23 cm^3, 1 M in ether, 0.23 mmol). The solution was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 18 hours. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give the hydrochloride salt **94** (0.0341 g, 100%) as a colourless oil, $[\alpha]_D$ +20.0 (1.35, CH_3OH); ν_{max} (neat)/cm^{-1} 3398, 2934, 1740, 1612, 1582, 1514 cm^{-1}; δ_H (250Hz; CD_3OD), 7.33 (2H, d, *J* 7.9, Ar*H*), 7.01 (2H, d, *J* 7.9, Ar*H*), 5.05 (1H, br s, C_3H), 4.15 (1H, br s, C_2H), 3.87 (3H, s, O*Me*), 3.73 (1H, m, $C_5H_XH_Y$), 3.19–3.04 (3H, m, CH_AH_BAr + CH_AH_BAr + $C_5H_XH_Y$), 2.63 (1H, br s, C_4H), 2.26 (3H, s, $CO\underline{CH_3}$), 1.26 (3H, d, *J* 6.7, CH*Me*); δ_c (62.9 MHz; CD_3OD) 170.2 (1C, Q), 159.4 (1C, Q), 130.0 (2C, CH), 127.8 (1C, Q), 114.4 (2C, CH), 78.5 (1C, CH), 62.9 (1C, CH), 54.7 (1C, CH_3) 50.1 (1C, CH_2) 39.3 (1C, CH), 31.4 (1C, CH_2), 19.8 (1C, CH_3), 16.3 (1C, CH_3); *m/z* (FAB) 264 ([M + H]$^+$, 100%), 204 (60), 142 (19), 121 (49); HRMS (FAB) (Found: [M + H]$^+$, 264.1593. $[C_{15}H_{22}NO_3]^+Cl^-$ requires *m/z* 264.1600).

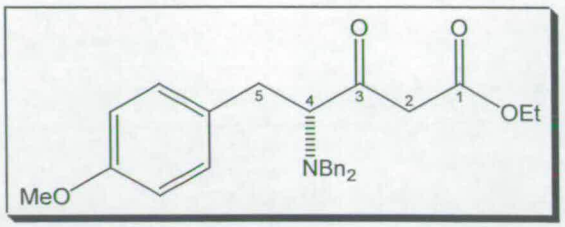
(2*R*)-2-Dibenzylamino-3-(4-methoxyphenyl)propanoic acid 104



To a solution of methyl ester **41** (1.97 g, 5.06 mmol) in tetrahydrofuran:water (30 cm^3 [4:1]) was added lithium hydroxide (1.06 g, 25.3 mmol). The solution was heated to reflux and held there for 18 hours, and then acidified to pH 2. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 25 cm^3). The combined organics were washed with water (20 cm^3), dried, and then concentrated under reduced pressure to give acid **104** (1.81 g, 95%) as a foam, which was used in the Claisen condensation reaction without further purification, R_f [hexane: EtOAc (7:3)] 0.3; $[\alpha]_D$ +25.8 (*c* 0.6, $CHCl_3$) [lit.(*ent*-**104**),[143] $[\alpha]_D$ –31.4 (*c* 1.0, $CHCl_3$)]; ν_{max} (neat)/cm^{-1} 3029, 2934, 2835, 1704, 1612, 1513; δ_H (360MHz; $CDCl_3$) 9.25 (1H, br s, O*H*), 7.29–7.25 (10H, m, Ar*H*), 7.11 (2H, d, *J* 8.4, Ar*H*), 6.82 (2H, d, *J* 8.4, Ar*H*), 4.00–3.82 (4H, m, $NCH_XH_YPh \times 2 + NCH_XH_YPh \times 2$), 3.86–3.83 (1H, m, C_2H), 3.78 (3H, s, O*Me*), 3.30 (1H, dd, *J* 14.5, 6.3, $C_3H_AH_B$). 3.17 (1H, dd, *J* 14.5, 8.5, $C_3H_AH_B$); δ_c (62.9 MHz; $CDCl_3$) 175.5 (1C, Q), 158.2 (1C, Q), 137.3 (2C, Q), 130.2 (2C, CH), 129.8 (1C, Q), 128.9 (4C, CH), 128.4 (4C, CH), 127.5 (2C, CH), 113.7 (2C, CH), 62.9 (1C, CH), 55.2 (1C, CH_3), 54.4 (2C, CH_2), 33.3 (1C, CH_2); *m/z* (FAB) 367 ($[M + H]^+$, 83%), 330 (22), 254 (40), 154 (38), 91, (100); HRMS (FAB) (Found: $[M + H]^+$, 376.1903. $C_{24}H_{26}NO_3$ requires *m/z* 376.1913).

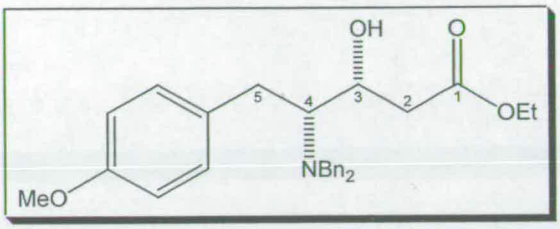
All spectroscopic data was in good agreement with that of the literature.[143]

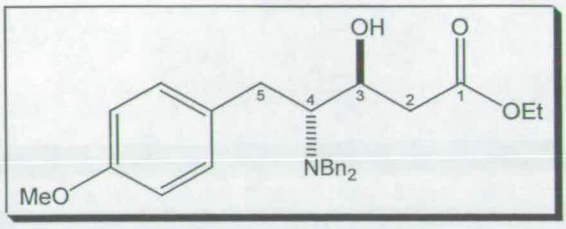
Ethyl (4*R*)-4-dibenzylamino-5-(4-methoxyphenyl)-3-oxopentanoate 99



To a solution of the acid **104** (1.81 g, 4.83 mmol) in THF (20 cm^3) was added carbonyldiimidazolide (1.56 g, 9.65 mmol). The mixture was stirred for 2.5 hours, and then cooled to -78 °C. Meanwhile a separate flask containing ethyl acetate (1.65 cm^3, 1.49 g, 16.9 mmol) was cooled to -78 °C, and lithium hexamethyldisilazide, (15.9 cm^3, 1.06 M in THF, 16.9 mmol) was added. The solution was stirred at -78 °C for 20 minutes and then transferred to the flask containing the imidazole *via* cannula. The combined solution was stirred at -78 °C for 2 hours and then quenched by the addition of 1% HCl (20 cm^3) and diluted with CH_2Cl_2 (20cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 75 cm^3); the combined organics were washed sequentially with water (100 cm^3) and brine (100 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (95:5)] to give **99** (1.76 g, 82%) as a white solid, R_f [hexane:EtOAc (10:1)] 0.32; mp 83–85 °C; $[\alpha]_D$ +75.3 (*c* 1.0, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 2936, 1745, 1716, 1612, 1584, 1513; δ_H (360MHz; $CDCl_3$) 7.36–7.19 (10H, m, Ar*H*), 7.05 (2H, d, *J* 8.7, Ar*H*), 6.78 (2H, d, *J* 8.7, Ar*H*), 4.01 (2H, q, *J* 7.2, O$CH_2$$CH_3$), 3.83 (2H, d, *J* 13.5, NC$H_X$$H_Y$Ph × 2), 3.76 (3H, s, O*Me*), 3.65 (1H, d, *J* 15.8, $C_2H_A$$H_B$), 3.59 (1H, dd, *J* 9.3, 3.7, C_4H), 3.54 (2H, d, *J* 13.5, $NCH_X$$H_Y$Ph × 2), 3.34 (1H, d, *J* 15.8, $C_2H_A$$H_B$), 3.13 (1H, dd, *J* 13.6, 9.3, $C_5H_S$$H_T$), 2.89 (1H, dd, *J* 13.6, 3.7, $C_5H_S$$H_T$), 1.09 (3H, t, *J* 7.2, OCH_2CH_3); δ_c (62.9 MHz; $CDCl_3$) 202.2 (1C, Q), 167.1 (1C, Q), 157.8 (1C, Q), 138.6 (2C, Q), 130.9 (1C, Q), 130.3 (2C, CH), 128.9 (4C, CH), 128.4 (4C, CH), 127.3 (2C, CH), 113.7 (2C, CH), 68.3 (1C, CH), 60.9 (1C, CH_2), 55.1 (1C, CH_3), 54.4 (2C, CH_2), 46.6 (1C, CH_2), 27.4 (1C, CH_2), 13.8 (1C, CH_3); (Found : C, 75.19; H, 6.83; N, 3.11. $C_{28}H_{31}NO_4$ requires C, 75.51; H, 6.97; N, 3.15%).

Ethyl (3*R*,4*R*)-4-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentanoate 98 and Ethyl (3*S*,4*R*)-4-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentanoate 105





To a solution of β-keto ester **99** (1.54, 3.47 mmol) in ether (30 cm^3) was added methanol (6.59 cm^3, 5.21 g, 163 mmol). The solution was adjusted to pH 4 by the addition of a few drops acetic acid, and then cooled to 0 °C. Sodium cyanoborohydride (2.15 g, 34.6 mmol) was added cautiously and the mixture was allowed to warm to room temperature. The solution was stirred for 18 hours and then quenched by the addition of a saturated aqueous solution of ammonium chloride (20 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 75 cm^3). The combined organics were dried, and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [hexane:EtOAc (9:1)] to give **98** (1.28 g, 83%) and **105** (0.0307 g, 2%) both as oils,

Data for (3*R*,4*R*) diastereomer

98: obtained (1.28 g, 83%) as a colourless oil,

R_f [hexane:EtOAc (10:1)] 0.38; $[\alpha]_D$ -36.0 (*c* 2.2, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3591, 3025, 2933, 1731, 1611, 1581, 1511; δ_H (360MHz; $CDCl_3$) 7.35–7.25 (10H, m, Ar*H*), 7.11 (2H, *J* 8.5, Ar*H*), 6.88 (2H, d, *J* 8.5, Ar*H*) 4.11–4.05 (2H, m, NCH_AH_BPh × 2), 4.08 (2H, q, *J* 7.2, OCH_2CH_3), 4.01–3.98 (1H, m, C_3H), 3.81 (3H, s, O*Me*), 3.44 (2H, d, *J* 13.4, NCH_AH_BPh × 2), 3.11–3.09 (1H, m, C_4H), 2.82–2.74 (2H, m, $C_5H_XH_Y$ + $C_5H_XH_Y$), 2.39 (1H, dd, *J* 15.7, 9.6, $C_2H_SH_T$), 2.12 (1H, br d, *J* 15.7, $C_2H_SH_T$), 1.21 (3H, t, *J* 7.2, OCH_2CH_3); δ_c (62.9 MHz; $CDCl_3$) 172.7 (1C, Q), 157.9 (1C, Q), 139.1 (2C, Q), 131.7 (1C, Q), 129.9 (2C, CH), 128.9 (4C, CH), 128.3 (4C,

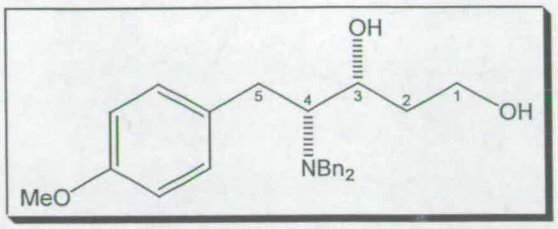
CH), 127.1 (2C, CH), 113.9 (2C, CH), 67.8 (1C, CH), 62.9 (1C, CH), 60.4 (1C, CH_2), 55.1 (1C, CH_3), 54.3 (2C, CH_2), 39.7 (1C, CH_2), 29.9 (1C, CH_2), 14.0 (1C, CH_3); *m/z* (FAB) 448 ($[M + H]^+$, 97%), 330 (88), 326 (89), 121 (37), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 448.2480. $C_{28}H_{34}NO_4$ requires *m/z* 448.2488); Chiral HPLC (*R* enantiomer) R_t = 16.0 minutes, (*S* enantiomer) R_t = 24.4 minutes [hexane-propan-2-ol (19 : 1)], >98% ee.

Data for (3*S*,4*R*) diastereomer **105**:

Obtained (0.0307 g, 2%) as a colourless oil,

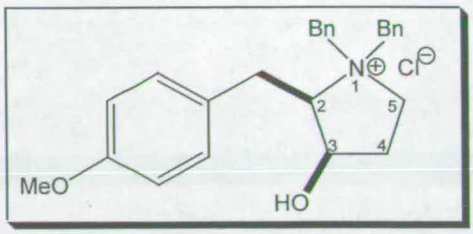
R_f [hexane:EtOAc (10:1)] 0.34; $[\alpha]_D$ -25.9 (*c* 0.81, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3501, 2934, 2833, 1728, 1612, 1584, 1512; δ_H (200 MHz; $CDCl_3$) 7.39–7.25 (10H, m, Ar*H*), 7.20 (2H, d , *J* 8.8, Ar*H*) 6.92 (2H, d, *J* 8.8, Ar*H*) 4.33–4.25 (1H, m, C_3H), 4.18 (2H, q, *J* 7.0, OCH_2CH_3), 3.90 (3H, s, O*Me*), 3.82 (2H, d, *J* 13.7, NCH_AH_BPh × 2), 3.68 (2H, d, *J* 13.7, NCH_AH_BPh × 2), 3.19–2.97 (2H, m, $C_4H + C_5H_XH_Y$), 3.10 (1H, dd, *J* 9.0, 6.3, $C_5H_XH_Y$), 2.80 (1H, dd, *J* 16.4, 2.7, $C_2H_SH_T$), 2.38 (1H, dd, *J* 16.4, 9.8, $C_2H_SH_T$), 1.32 (3H, t, *J* 7.0, OCH_2CH_3); δ_c (62.9 MHz; $CDCl_3$) 173.4 (1C, Q), 158.0 (1C, Q), 139.7 (2C, Q), 133.1 (1C, Q), 130.5 (2C, CH), 128.9 (4C, CH), 128.4 (4C, CH), 127.1 (2C, CH), 113.9 (2C, CH), 69.2 (1C, CH), 63.0 (1C, CH), 60.8 (1C, CH_2), 55.5 (1C, CH_3), 54.8 (2C, CH_2), 39.7 (1C, CH_2), 31.5 (1C, CH_2), 14.3 (1C, CH_3); *m/z* (FAB) 448 ($[M + H]^+$, 88%), 330 (96), 326 (96), 121 (78), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 448.2489. $C_{28}H_{34}NO_4$ requires *m/z* 448.2488).

(3*R*,4*R*)-4-Dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentan-1-ol 97



A solution of ethyl ester **98** (0.697 g, 1.56 mmol) in THF (30 cm^3) was placed at -78 °C and lithium aluminium hydride (7.8 cm^3, 1.0 M in THF, 7.8 mmol) was added. The solution was stirred at -78 °C for 6 hours then quenched by the addition of 1 M aqueous sodium hydroxide (10 cm^3). The resulting mixture was allowed to warm to room temperature and diluted with CH_2Cl_2 (20 cm^3). Saturated aq. sodium potassium tartrate (40 cm^3) was added and the biphasic mixture was stirred vigorously for 15 hours by which time two clear phases were apparent. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 cm^3). The combined organics were dried, and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [hexane:EtOAc] (7:3 → 1:1)] to give **97** (0.598 g, 95%) as an oil, R_f [hexane:EtOAc (3:1)] 0.33; $[\alpha]_D$ -38.3 (*c* 1.7, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3388, 3028, 2933, 2835, 1612, 1583, 1512; δ_H (250 MHz; $CDCl_3$) 7.39–7.18 (10H, m, Ar*H*), 7.12 (2H, d, *J* 8.7, Ar*H*), 6.88 (2H, d, *J* 8.7, Ar*H*), 4.80 (1H, s, O*H*), 3.90 (2H, d, *J* 13.2, NC$H_A$$H_B$Ph × 2), 3.82 (3H, s, O*Me*), 3.77 (1H, dd, *J* 8.9, 2.7, C_3H), 3.65 (2H, br s, CH_2OH), 3.39 (2H,d, *J* 13.2, NC$H_A$$H_B$Ph × 2), 3.08 (1H, dd, *J* 14.3, 6.4, $C_5H_XH_Y$), 2.89 (1H, td, *J* 6.4, 2.7, C_4H), 2.60 (1H, dd, *J* 14.3, 6.4, $C_5H_XH_Y$), 1.82 (1H, s, C_3HO*H*), 1.65–155 (1H, m, $C_2H_SH_T$), 1.35–1.23 (1H, m, $C_2H_SH_T$); δ_c (62.9 MHz; $CDCl_3$) 158.0 (1C, Q), 138.5 (2C, Q), 131.8 (1C, Q), 129.9 (2C, CH), 128.9 (4C, CH), 128.4 (4C, CH), 127.2 (2C, CH), 113.9 (2C, CH), 70.5 (1C, CH), 63.8 (1C, CH), 61.2 (1C, CH_2), 55.1 (1C, CH_3), 53.6 (2C, CH_2), 35.6 (1C, CH_2), 31.1 (1C, CH_2); *m/z* (FAB) 406 ($[M + H]^+$, 69%), 330 (71), 284 (69), 181 (45), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 406.2387. $C_{26}H_{32}NO_3$ requires *m/z* 406.2382).

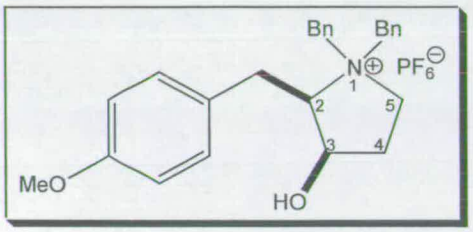
(2*R*,3*R*)-1,1-Dibenzyl-3-hydroxy-2-(4-methoxybenzyl)pyrrolidinium chloride 108



To a solution of the alcohol **97** (0.587 g, 1.45mmol) in CH_2Cl_2 (20 cm^3) at 0 °C was added DMAP (0.292 g, 2.39 mmol) and triisopropylbenzene sulfonyl chloride (TIBSCl) (0.483 g, 1.59 mmol). The solution was stirred for 18 hours before being diluted with CH_2Cl_2 (20 cm^3) and water (25 cm^3). The organic phase was separated and washed with 1% HCl (2 × 30 cm^3) then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [CH_2Cl_2:MeOH (100:0) → (95:5)] to give a white foam. The salt obtained was subjected to ion exchange chromatography [Dowex Cl^-; prepared by treating Dowex 1-X2 with 1% aqueous hydrochloric acid followed by flushing with methanol until the eluent returned to pH 7] eluting with methanol to give the chloride salt **108** (0.520 g, 85%) as an amorphous solid, R_f [CH_2Cl_2:CH_3OH (95:5)] 0.07; $[\alpha]_D$ -55.2 (*c* 0.75, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3198, 1611, 1584, 1513; δ_H (360 MHz; $CDCl_3$) 7.78–7.29 (10H, m, Ar*H*), 7.33 (2H, d, *J* 8.5, Ar*H*), 6.74 (1H, s, O*H*), 6.67 (2H, d, *J* 8.5, Ar*H*), 5.72 (1H, br d, *J* 13.5, NC$H_X$$H_Y$Ph), 5.12 (1H, d, *J* 13.1, NC$H_A$$H_B$Ph), 5.07 (1H, d, *J* 13.1, NC$H_A$$H_B$Ph), 4.34 (1H, br s, C_3H), 4.28 (1H, d, *J* 13.5, NC$H_X$$H_Y$Ph), 3.97 (1H, br d, *J* 12.0, C$H_S$$H_T$Ar), 3.92–3.86 (1H, m, $C_5H_MH_N$), 3.69 (3H, s, O*Me*), 3.56 (1H, br t, *J* 12.0, C$H_S$$H_T$Ar), 3.39 (1H, br t, *J* 10.5, C_2H), 3.10 (1H, dt, 11.3, 8.3, $C_5H_MH_N$), 2.63 (1H, dt, 14.6, 7.3, $C_4H_EH_F$), 1.95–1.87 (1H, m, $C_4H_EH_F$); δ_c (62.9 MHz; $CDCl_3$) 158.9 (1C, Q), 134.1 (2C, CH), 133.6 (2C, CH), 131.2 (2C, CH), 131.1 (1C, CH), 130.8 (1C, Q), 129.8 (2C, CH), 129.6 (2C, CH), 128.6 (1C, CH), 128.0 (1C, Q), 127.9 (1C, Q), 114.5 (2C, CH), 76.7 (1C, CH), 68.2 (1C, CH), 63.1 (1C, CH_2), 62.4 (1C, CH_2), 56.3 (1C, CH_2), 55.5 (1C, CH_3), 31.9 (1C, CH_2) 28.1 (1C, CH_2); *m/z*

(FAB) 388 ($[M]^+$, 98%), 296 (48), 210 (24), 176 (71), 91 (100); HRMS (FAB) (Found: $[M]^+$, 338.2267. $[C_{26}H_{30}NO_2]^+Cl^-$ requires *m/z* 388.2277).

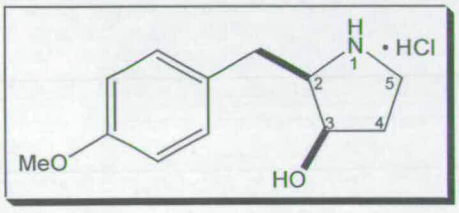
(2*R*,3*R*)-1,1-Dibenzyl-3-hydroxy-2-(4-methoxybenzyl)pyrrolidinium hexafluorophosphate 110



To a solution of the alcohol **97** (0.458 g, 1.13 mmol) in CH_2Cl_2 (20 cm^3) at 0 °C was added DMAP (0.228 g, 1.86 mmol) and TIBSCl (0.377 g, 1.24 mmol). The solution was stirred for 18 hours before being diluted with CH_2Cl_2 (20 cm^3) and water (25 cm^3). The organic phase was separated and washed with 1% HCl (2 × 30 cm^3) then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [CH_2Cl_2:MeOH (100:0) → (95:5)] to give a white foam. The salt obtained was subjected to ion exchange chromatography [Dowex PF_6^-; prepared by treating Dowex 1-X2 with 1% aqueous hexafluorophosphoric acid followed by flushing with methanol until the eluent returned to pH 7] eluting with methanol to give the hexafluoride salt **110** (0.420 g, 70%) as a white solid. Recrystallisation from methanol provided an analytical sample, R_f [CH_2Cl_2:CH_3OH (95:5)] 0.05; mp 211–212 °C; $[\alpha]_D$ -38.44 (*c* 1.9, $(CH_3)_2CO$); ν_{max} (KBr)/cm^{-1} 3581, 3419, 2962, 1613, 1586, 1513; δ_H (360 MHz; $(CD_3)_2CO$) 7.87–7.55 (10H, m, Ar*H*), 7.40 (2H, d, *J* 8.8, Ar*H*), 6.95 (2H, d, *J* 8.8, Ar*H*), 5.28 (1H, d, *J* 13.3, NCH_XH_YPh), 5.18 (1H, d, *J* 13.4, NCH_SH_TPh), 4.89 (1H, d, *J* 13.3, NCH_XH_YPh), 4.69 (1H, d, *J* 13.4, NCH_SH_TPh), 4.50 (1H, br s, C_3H), 4.00 (1H, ddd, *J* 12.6, 8.4, 3.3, $C_5H_EH_F$), 3.82 (3H, s, O*Me*), 3.73 (3H, br s, CH_MH_NAr + CH_MH_NAr + C_2H), 3.57 (1H, br q, *J* 11.1, $C_5H_EH_F$), 2.65–2.56 (1H, m, $C_4H_AH_B$), 2.42 (1H, dddd, *J* 12.7, 11.9, 8.4, 2.4 $C_4H_AH_B$); δ_c (62.9

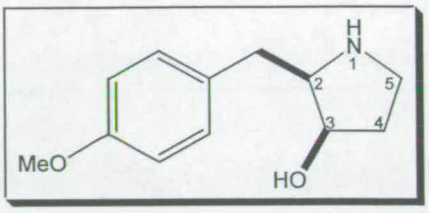
MHz; $(CD_3)_2CO$) 158.1 (1C, Q), 132.6 (2C, CH), 132.4 (2C, CH), 129.9 (1C, CH), 129.8 (2C, CH), 129.4 (2C, CH), 128.5 (1C, CH), 128.4 (2C, CH), 127.2 (1C, Q), 126.5 (1C, Q), 126.3 (1C, Q), 113.2 (2C, CH), 73.9 (1C, CH), 66.6 (1C, CH), 60.6 (1C, CH_2), 60.1 (1C, CH_2), 53.9 (1C, CH_2), 53.8 (1C, CH_3), 30.6 (1C, CH_2), 25.7 (1C, CH_2); *m/z* (FAB) 388 ($[M]^+$, 100%), 296 (11), 176 (24), 154 (47), 136 (41), 91 (75); HRMS (FAB) (Found: $[M]^+$, 338.2281. $[C_{26}H_{30}NO_2]^+PF_6^-$ requires *m/z* 388.2277).

(2*R*,3*R*)-3-Hydroxy-2-(4-methoxybenzyl)pyrrolidine hydrochloride 109



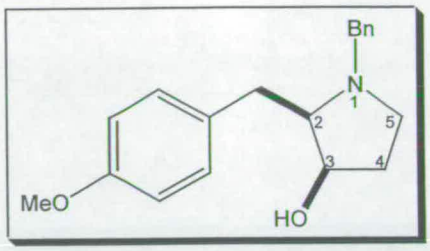
A solution of the chloride salt **108** (0.243 g, 0.574 mmol) and Pearlman's catalyst [0.243 g; 20% $Pd(OH)_2/C$] in methanol (7 cm^3) was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 24 hours. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give **109** (0.133 g, 95%) as a white solid, mp 261–263 °C; $[\alpha]_D$ +15.2 (*c* 1.12, CH_3OH); ν_{max} (KBr)/cm^{-1} 3330, 3302, 2955, 1614, 1556, 1516; δ_H (250MHz; CD_3OD) 7.35 (2H, d, *J* 8.7, Ar*H*), 6.99 (2H, d, *J* 8.7, Ar*H*), 4.39–4.36 (1H, m, C_3H), 3.87 (3H, s, O*Me*), 3.67 (1H, ddd, *J* 8.5, 6.6, 2.8, C_2H), 3.55 (1H, ddd, *J* 11.5, 9.9, 8.2, $C_5H_SH_T$), 3.57–3.23 (1H, m, $C_5H_SH_T$), 3.24 (1H, dd, *J* 14.2, 6.6, CH_XH_YAr), 3.02 (1H, dd, *J* 14.2, 8.5, CH_XH_YAr), 2.32 – 2.10 (2H, m, $C_4H_MH_N + C_4H_MH_N$); δ_c (62.9 MHz; CD_3OD) 158.4 (1C, Q), 129.2 (2C, CH), 128.0 (1C, Q), 113.4 (2C, CH), 69.1 (1C, CH), 66.0 (1C, CH), 53.8 (1C, CH_3), 42.3 (1C, CH_2), 32.2 (1C, CH_2), 30.8 (1C, CH_2); *m/z* (FAB) 208 ($[M + H]^+$, 97%), 154 (100), 136 (85), 121 (15); HRMS (FAB) (Found: $[M + H]^+$, 208.1340. $[C_{12}H_{18}NO_2]^+Cl^-$ requires *m/z* 208.1338).

(2*R*,3*R*)-3-Hydroxy-2-(4-methoxybenzyl)pyrrolidine 85



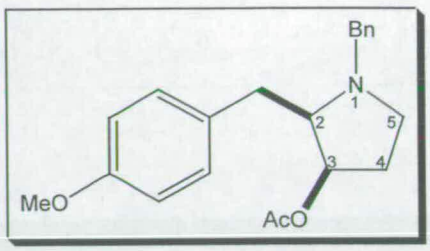
The HCl salt **109** was subjected to ion-exchange chromatography [Dowex OH^-] (prepared by treating Dowex 1-X2 with 1 M aqueous NaOH, followed by methanol until the pH of the eluent returned to 7). Eluting with methanol gave the free base (quantitative recovery) as a white solid, R_f [CH_2Cl_2: MeOH (95:5)] 0.06; mp 127–128 °C; $[\alpha]_D$ -30.9 (*c* 1.36, CH_3OH); ν_{max} (KBr)/cm^{-1} 3426, 3290, 2961, 1612, 1583, 1512; δ_H (250 MHz; CD_3OD) 7.23 (2H, d, *J* 8.7, Ar*H*), 6.87 (2H, d, *J* 8.7, Ar*H*), 4.06 (1H, ddd, *J* 4.9, 3.1, 1.5, C_3H), 3.79 (3H, s, O*Me*), 3.17 (1H, ddd, *J* 11.1, 8.5, 7.5, $C_5H_AH_B$), 2.96 (1H, dd, *J* 7.1, 3.2, CH_XH_YAr), 2.90 (1H, dd, *J* 7.1, 2.5, CH_XH_YAr), 2.79 (1H, ddd, *J* 11.1, 9.7, 4.7, $C_5H_AH_B$), 2.85–2.67 (1H, m, C_2H), 2.01 (1H, dddd, *J* 13.7, 9.7, 7.5, 4.9, $C_4H_MH_N$), 1.83 (1H, dddd, *J* 13.7, 8.5, 4.7, 1.5, $C_4H_MH_N$); δ_c (62.9 MHz; CD_3OD) 158.6 (1C, Q), 132.1 (1C, Q), 130.0 (2C, CH), 113.8 (2C, CH), 71.9 (1C, CH), 66.2 (1C, CH), 54.6 (1C, CH_3), 43.6 (1C, CH_2), 35.0 (1C, CH_2), 34.1 (1C, CH_2); *m/z* (FAB) 208 ($[M + H]^+$, 100%), 190 (11), 121 (27), 91 (69); HRMS (FAB) (Found: $[M + H]^+$, 208.1333. $C_{12}H_{18}NO_2$ requires *m/z* 208.1338).

(2*R*,3*R*)-1-Benzyl-3-hydroxy-2-(4-methoxybenzyl)pyrrolidine 96



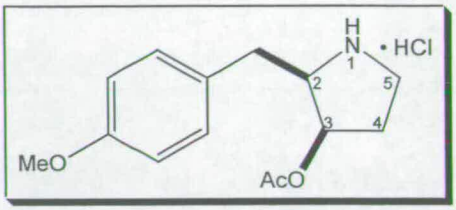
To a solution of the chloride salt **108** (0.269 g, 0.634 mmol) in methanol (10 cm^3) was added 5% Pd/C (0.027 g) and potassium carbonate (0.263 g, 1.90 mmol). The mixture was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 20 minutes. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. To the residue was added CH_2Cl_2 (25 cm^3) and water (25 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 25 cm^3); the combined organic phase were washed with water (25 cm^3) and then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (1:1)] to give pyrrolidine **96** (0.150 g, 80%) as an oil, R_f [hexane:EtOAc (1:1)] 0.36; $[\alpha]_D$ -104.0 (*c* 1.18, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3418, 2934, 1611, 1583, 1512; δ_H (250 MHz; $CDCl_3$) 7.41–7.26 (7H, m, Ar*H*), 6.89 (2H, d, *J* 8.7, Ar*H*), 4.20 (1H, d, *J* 13.0, NC$H_A$$H_B$Ph), 3.96 (1H, br s, C_3H), 3.83 (3H, s, O*Me*), 3.26 (1H, d, *J* 13.0, NC$H_A$$H_B$Ph), 3.05 (1H, dt, *J* 8.8, 3.7, C_2H), 2.98–2.86 (2H, m, C$H_S$$H_T$Ar + C$H_S$$H_T$Ar), 2.49 (1H, ddd, *J* 8.5, 6.0, 3.7, $C_5H_MH_N$), 2.20–2.06 (1H, m, $C_5H_MH_N$), 1.99 (1H, ddd, 13.7, 6.4, 3.7 $C_4H_XH_Y$), 1.75–1.63 (1H, m, $C_4H_XH_Y$); δ_c (62.9 MHz; $CDCl_3$) 158.4 (1C, Q), 139.4 (1C, Q), 131.9 (1C, Q), 130.7 (2C, CH), 129.3 (2C, CH), 128.7 (2C, CH), 127.4 (1C, CH), 114.2 (2C, CH), 72.5 (1C, CH), 71.3 (1C, CH), 58.3 (1C, CH_2), 55.7 (1C, CH_3), 51.9 (1C, CH_2), 33.4 (1C, CH_2), 32.7 (1C, CH_2); *m/z* (FAB) 298 ($[M + H]^+$, 90%), 176 (93), 154 (62), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 298.1805. $C_{19}H_{24}NO_2$ requires *m/z* 298.1805).

(2*R*,3*R*)-3-Acetoxy-1-benzyl-2-(4-methoxybenzyl)pyrrolidine 111



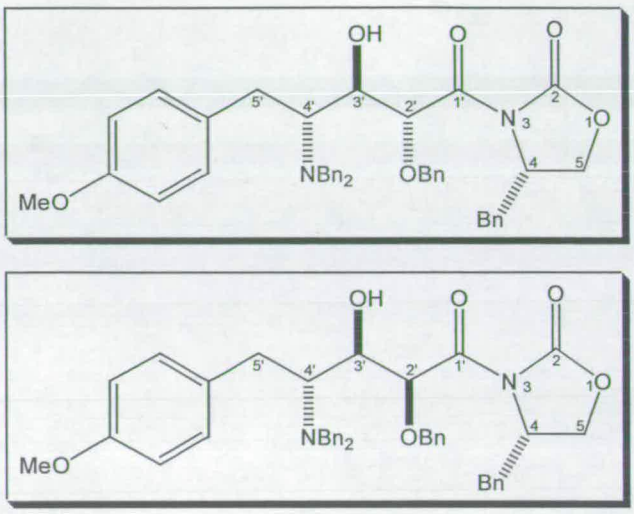
To a solution of the alcohol **96** (0.0643 g, 0.216 mmol) in CH_2Cl_2 (5 cm^3) was added freshly distilled acetic anhydride (0.044 g, 0.041 cm^3, 0.43 mmol), and triethylamine (0.044 g, 0.061 cm^3, 0.43 mmol). The solution was stirred for 18 hours and then quenched by the addition of saturated aq. sodium bicarbonate (20 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was dried and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (7:3)] to give the ester **111** (0.0638g, 87%) as a colourless oil, R_f [hexane:EtOAc (1:1)] 0.58; $[\alpha]_D$ -123.1 (*c* 0.26, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2935, 2791, 1733, 1612, 1581, 1513; δ_H (250 MHz; $CDCl_3$) 7.40–7.33 (5H, m, ArH), 7.22 (2H, d, *J* 8.7, ArH), 6.86 (2H, d, *J* 8.7, ArH), 5.01 (1H, ddd, *J* 8.8, 5.1, 3.4, C_3H), 4.12 (1H, d, *J* 13.1, NCH_AH_BPh), 3.39 (3H, s, O*Me*), 3.36 (1H, d, *J* 13.1 NCH_AH_BPh), 2.97 (1H, dd, *J* 8.7, 1.8, $C_5H_XH_Y$), 2.94 (1H, dd, *J* 13.3, 5.1, CH_SH_TAr), 2.78 (1H, dd, *J* 13.3, 9.5, CH_SH_TAr), 2.66 (1H, dt, *J* 9.5, 5.1, C_2H), 2.17 (2H, m, $C_4H_MH_N$ + $C_5H_XH_Y$), 2.14 (3H, s, $COCH_3$), 1.69–1.54 (1H, m, $C_4H_MH_N$); δ_c (62.9 MHz; $CDCl_3$) 171.1 (1C, Q), 158.4 (1C, Q), 138.8 (1C, Q), 131.5 (1C, Q), 130.2 (2C, CH), 129.6 (2C, CH), 128.7 (2C, CH), 127.5 (1C, CH), 114.3 (2C, CH), 75.2 (1C, CH), 68.8 (1C, CH), 58.8 (1C, CH_2), 55.7 (1C, CH_3), 52.1 (1C, CH_2), 33.8 (1C, CH_2), 31.1 (1C, CH_2), 21.7 (1C, CH_3); *m/z* (FAB) 340 ($[M + H]^+$, 70%), 280 (28), 218 (52), 121 (43), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 340.1916. $C_{21}H_{26}NO_3$ requires *m/z* 340.1913).

(2*R*,3*R*)-3-Acetoxy-2-(4-methoxybenzyl)pyrrolidine hydrochloride 112



To a solution of the pyrrolidine **111** (0.0527 g, 0.155 mmol) and Pearlman's catalyst [0.0527 g; 20% $Pd(OH)_2/C$] in methanol (5 cm^3) was added hydrochloric acid [0.31 cm^3 (1 M in ether), 0.31 mmol]. The solution was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 20 hours. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give **112** (0.0450 g, 100%) as a pale brown oil. $[\alpha]_D$ -46.3 (*c* 0.56, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3398, 2934, 1741, 1613, 1581, 1515; δ_H (250 MHz; CD_3OD) 7.32 (2H, d, *J* 8.4, Ar*H*), 7.00 (2H, d, *J* 8.4, Ar*H*), 5.40 (1H, br s, C_3H), 4.02 (1H, br s, C_2H), 3.86 (3H, s, O*Me*), 3.54 (2H, br q, *J* 8.6, $C_5H_SH_T + C_5H_SH_T$), 3.18 (1H, dd, *J* 14.1, 6.2, CH_AH_BAr), 3.06 (1H, dd, *J* 14.1, 8.4, CH_AH_BAr), 2.52–2.44 (1H, m, $C_4H_MH_N$), 2.31–2.18 (1H, m, $C_4H_MH_N$), 2.27 (3H, s, $COCH_3$); δ_c (62.9 MHz; CD_3OD) 169.3 (1C, Q), 158.6 (1C, Q), 129.1 (2C, CH), 127.1 (1C, Q), 113.6 (2C, CH), 72.4 (1C, CH), 64.1 (1C, CH), 53.8 (1C, CH_3), 42.4 (1C, CH_2), 30.7 (1C, CH_2), 29.9 (1C, CH_2), 19.0 (1C, CH_3); *m/z* (FAB) 250 ($[M + H]^+$, 100%), 190 (36), 128 (22), 121 (32), 91 (41); HRMS (FAB) (Found: $[M + H]^+$, 250.1442. $[C_{14}H_{20}NO_3]^+Cl^-$ requires *m/z* 250.1443).

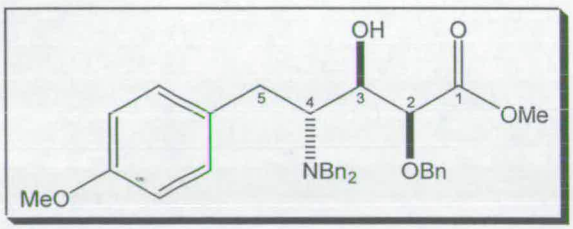
(2'*R*,3'*R*,4*S*,4'*R*)-3-[2'-Benzyloxy-4'-*N*,*N*-dibenzylamino-3'-hydroxy-5'-(4-methoxyphenyl)-1'-oxopentyl]-4-phenylmethyloxazolidin-2-one 156 and (2'*S*,3'*R*,4*S*,4'*R*)-3-[2'-Benzyloxy-4'-*N*,*N*-dibenzylamino-3'-hydroxy-5'-(4-methoxyphenyl)-1'-oxopentyl]-4-phenylmethyloxazolidin-2-one 155

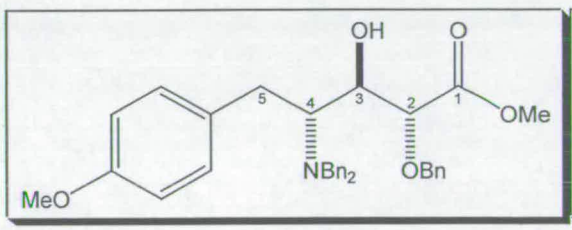


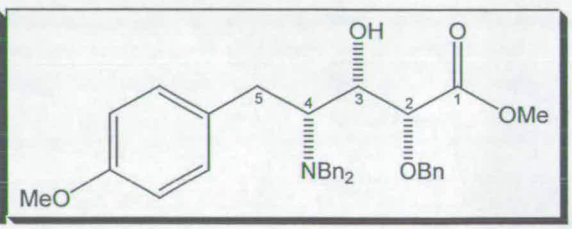
Synthesised in analogous manner to **30** from *S*-imide *ent*-**32** to give two diastereomers one of which **155** was inseparable from the imide *ent*-**32**. Thus glycolate equivalent *ent*-**32** (0.660 g, 2.03 mmol), dibutylboron triflate (2.5 cm^3, 1.0 M in CH_2Cl_2, 2.5 mmol), and triethylamine (0.27 g, 0.37 cm^3, 2.7 mmol) in CH_2Cl_2 (3 cm^3), along with aldehyde **31** (0.200 g, 0.557 mmol) in CH_2Cl_2 (3 cm^3) gave, after chromatography using preparative HPLC [hexane:EtOAc (7:3)], **156** (0.145 g, 38%) as an oil, R_f [hexane: EtOAc (7:3)] 0.40; R_t [hexane:EtOAc (7:3)] = 11.98 minutes; $[\alpha]_D$ +42.2 (*c* 1.39, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3516, 3029, 1777, 1707, 1611, 1584, 1512; δ_H (360 MHz; $CDCl_3$) 7.39–7.05 (20H, m, Ar*H*), 6.87 (2H, d, *J* 8.7, Ar*H*), 6.77 (2H, d, *J* 8.7, Ar*H*), 5.08 (1H, d, *J* 9.2, $C_{2'}H$), 4.50–4.43 (1H, m, C_4H), 4.30 (1H, d, *J* 11.7, OCH_AH_BPh), 4.25 (1H, *J* 11.7, OCH_AH_BPh), 4.19 – 4.16 (1H, m, $C_{3'}H$), 4.14 (1H, dd, *J* 9.1, 2.4, $C_5H_XH_Y$), 4.07 (1H, dd, *J* 9.1, 7.6, $C_5H_XH_Y$), 3.91 (2H, d, *J* 14.2, NCH_SH_TPh), 3.84 (3H, s, O*Me*), 3.62 (2H, d, *J* 14.2, NCH_SH_TPh), 3.33 (1H, dd, *J* 13.4, 3.3, $C_4HCH_MH_NPh$), 3.23 (1H, br t, *J* 7.0, $C_{4'}H$), 2.88 (2H, d, *J* 7.1, $C_{5'}H_EH_F$ + $C_{5'}H_EH_F$), 2.65 (1H, dd, *J* 13.4, 9.9, $C_4HCH_MH_NPh$); δ_c (62.9 MHz, $CDCl_3$) 172.7 (1C, Q), 157.7 (1C, Q), 154.3 (1C, Q), 139 (2C, Q),

136.5 (1C, Q), 135.0 (1C, Q), 132.0 (1C, Q), 130.4 (2C, CH), 129.3 (2C, CH), 128.8 (2C, CH), 128.7 (2C, CH), 128.6 (4C, CH), 128.2 (2C, CH), 127.9 (5C, CH), 127.2 (1C, CH), 126.4 (2C, CH), 113.2 (2C, CH), 76.9 (1C, CH), 72.5 (1C, CH_2), 71.7 (1C, CH), 66.7 (1C, CH_2), 60.1 (1C, CH), 55.7 (1C, CH), 55.2 (1C, CH_3), 54.0 (2C, CH_2), 37.3 (1C, CH_2), 30.1 (1C, CH_2); *m/z* (FAB) 685 ($[M + H]^+$, 26%), 683 (30), 330 (100), 563 (15), 121 (44), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 685.3279. $C_{43}H_{45}N_2O_6$ requires *m/z* 685.3278).

Methyl (2*S*,3*R*,4*R*)-2-benzyloxy-4-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentanoate 148 and Methyl (2*R*,3*R*,4*R*)-2-benzyloxy-4-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentanoate 149 and Methyl (2*R*,3*S*,4*R*)-2-benzyloxy-4-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentanoate 150







To a solution of diisopropylethylamine (0.46 cm^3, 0.34 g, 2.6 mmol) and dibutylboron triflate (2.46 cm^3, 1.0 M in CH_2Cl_2, 2.46 mmol) in CH_2Cl_2 at -78 °C, was added a solution of methyl ester **134** (0.366 g, 2.03 mmol) in CH_2Cl_2 (3 cm^3) *via* cannula. The resulting solution was allowed to stir for 1.5 hours before a -78 °C solution of amino aldehyde **31** (0.200 g, 0.557 mmol) in CH_2Cl_2 (3 cm^3) was added, again, *via* cannula. The mixture produced was left to stir at -78 °C for 2 hours. The reaction was quenched by the addition of methanol (5 cm^3) followed by pH 7

phosphate buffer (5 cm^3). Hydrogen peroxide (30% aq. solution; 5 cm^3 in methanol (10 cm^3) was added dropwise to the solution and the mixture was stirred and warmed to room temperature over *ca.* 1 hour. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was washed sequentially with saturated aq. sodium bicarbonate (30 cm^3) and brine (30 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed using preparative HPLC [hexane:EtOAc (7:3)] to give **148** (0.1245 g, 42%), **149** (0.0607 g, 20%) and **150** (0.0283 g, 9%) all as oils.

Data for **(2*S*,3*R*,4*R*)** diastereomer

148: obtained (0.1245 g, 42%) as a colourless oil,

R_f [hexane:EtOAc (7:3)] 0.57; R_t [hexane:EtOAc (7:3)] = 13.78 minutes; $[\alpha]_D$ -27.3 (*c* 5.77, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3557, 2951, 1751, 1611, 1512; δ_H (360 MHz; $CDCl_3$) 7.41–7.11 (15H, m, Ar*H*), 7.17 (2H, d, *J* 8.6, Ar*H*), 6.86 (2H, d, *J* 8.6, Ar*H*), 4.43 (1H, d, *J* 10.6, OCH_AH_BPh), 4.18 (1H, br s, C_2H), 4.16 (1H, br d, *J* 5.5, C_3H), 3.84 (3H, s, O*Me*), 3.79 (2H, d, *J* 13.7, NCH_XH_YPh × 2), 3.71 (1H, d, *J* 10.6, OCH_AH_BPh), 3.66 (3H, s, O*Me*), 3.64 (2H, d, *J* 13.7, NCH_XH_YPh × 2), 3.22 (1H, q, *J* 6.0, C_4H), 3.10 (1H, dd, *J* 14.2, 6.6, $C_5H_MH_N$), 2.98 (1H, dd, *J* 14.2, 6.5, $C_5H_MH_N$), 2.29 (1H, br s, O*H*); δ_c (62.9 MHz; $CDCl_3$) 171.7 (1C, Q), 157.6 (1C, Q), 139.8 (2C, Q), 136.9 (1C, Q), 132.9 (1C, Q), 130.2 (2C, CH), 129.0 (4C, CH), 128.1 (4C, CH), 128.0 (2C, CH), 127.9 (2C, CH), 127.6 (1C, CH), 126.8 (2C, CH), 113.5 (2C, CH), 78.2 (1C, CH), 73.4 (1C, CH), 72.3 (1C, CH_2), 60.0 (1C, CH), 55.1 (1C, CH_3), 54.1 (2C, CH_2), 51.8 (1C, CH_3), 30.9 (1C, CH_2); *m/z* (FAB) 540 ($[M + H]^+$, 35%), 418 (54), 330 (63), 121 (37), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 540.2736. $C_{34}H_{38}NO_5$ requires *m/z* 540.2750).

Data for **(2*R*,3*R*,4*R*)** diastereomer

149: obtained (0.0607 g, 29%) as a colourless oil,

R_f [hexane:EtOAc (7:3)] 0.53; R_t [hexane:EtOAc (7:3)] = 18.14 minutes; $[\alpha]_D$ +0.63 (*c* 1.88, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3504, 3028, 2951, 1739, 1612, 1584, 1512; δ_H (360 MHz; $CDCl_3$) 7.44–7.14 (15H, m, Ar*H*), 7.00 (2H, d, *J* 8.6, Ar*H*), 6.86 (2H, d, *J* 8.6, Ar*H*), 4.66 (1H, d, *J* 11.6, OC$H_A$$H_B$Ph), 4.37 (1H, dd, *J* 7.2, 2.6 C_3H), 4.23 (1H, d, *J* 11.6, OC$H_A$$H_B$Ph), 3.95 (1H, d, *J* 7.2, C_2H), 3.91 (3H, s, O*Me*), 3.89 (2H, d, *J* 14.4, NC$H_X$$H_Y$Ph × 2), 3.74 (3H, s, O*Me*), 3.68 (2H, d, *J* 14.4, NC$H_X$$H_Y$Ph × 2), 3.27 (1H, ddd, *J* 8.5, 5.5, 2.6, C_4H), 2.98 (1H, dd, *J* 14.2, 8.5, $C_5H_MH_N$), 2.89 (1H, dd, *J* 14.2, 5.5, $C_5H_M$$H_N$), 2.54 (1H, br s, O*H*); δ_c (62.9 MHz; $CDCl_3$) 172.4 (1C, Q), 158.3 (1C, Q), 140.4 (2C, Q), 137.1 (1C, Q), 132.9 (1C, Q), 131.0 (2C, CH), 129.1 (4C, CH), 128.9 (2C, CH), 128.7 (2C, CH), 128.5 (4C, CH), 128.4 (1C, CH), 127.1 (2C, CH), 113.9 (2C, CH), 80.0 (1C, CH), 73.0 (1C, CH_2), 71.3 (1C, CH), 60.3 (1C, CH), 55.8 (1C, CH_3), 54.8 (2C, CH_2), 52.5 (1C, CH_3), 31.2 (1C, CH_2); *m/z* (FAB) 540 ($[M + H]^+$, 65%), 538 (52), 418 (55), 330 (64), 121 (58), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 540.2747. $C_{34}H_{38}NO_5$ requires *m/z* 540.2750).

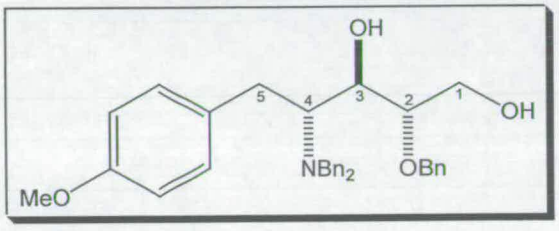
Data for **(2*R*,3*S*,4*R*)** diastereomer

150: obtained (0.0283 g, 9%) as a colourless oil,

R_f [hexane:EtOAc (7:3)] 0.45; R_t [hexane:EtOAc (7:3)] = 19.45 minutes; $[\alpha]_D$ -18.03 (*c* 0.92, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3357, 3029, 2951, 2837, 1755, 1611, 1583, 1512; δ_H (250 MHz; $CDCl_3$) 7.49–6.88 (15H, m, Ar*H*), 7.05 (2H, d, *J* 8.6, Ar*H*), 6.74 (2H, d, *J* 8.6, Ar*H*), 4.53 (1H, d, *J* 11.7, OC$H_A$$H_B$Ph), 4.14 (1H, dd, *J* 9.1, 2.1, C_3H), 4.03 (2H, d, *J* 13.3, NC$H_X$$H_Y$Ph × 2), 3.82 (3H, s, O*Me*), 3.80 (3H, s, O*Me*), 3.70 (1H, d, *J* 2.1, C_2H), 3.59–3.43 (1H, m, C_4H), 3.53 (2H, d, *J* 13.3, NC$H_X$$H_Y$Ph × 2), 3.48 (1H, d, *J* 11.7, OC$H_A$$H_B$Ph), 3.24 (1H, dd, *J* 14.4, 5.0, $C_5H_MH_N$), 2.65 (1H, dd, *J* 14.4, 7.9, $C_5H_M$$H_N$); δ_c (62.9 MHz; $CDCl_3$) 171.6 (1C, Q), 158.0 (1C, Q), 138.2 (2C, Q), 137.6 (1C, Q), 131.2 (1C, Q), 129.9 (2C, CH), 128.9 (4C, CH), 128.5 (4C, CH), 127.9 (2C,

CH), 127.2 (2C, CH), 126.9 (3C, CH), 113.8 (2C, CH), 78.1 (1C, CH), 72.5 (1C, CH), 71.6 (1C, CH_2), 59.0 (1C, CH), 55.1 (1C, CH_3), 53.7 (2C, CH_2), 52.0 (1C, CH_3), 31.3 (1C, CH_2); *m/z* (FAB) 540 ($[M + H]^+$, 89%), 450 (63), 418 (47), 330 (53), 181 (40), 121 (65), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 540.2757. $C_{34}H_{38}NO_5$ requires *m/z* 540.2750).

(2*S*,3*R*,4*R*)-Benzyloxy-4-*N*,*N*-dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentan-1-ol 151



*Via reduction of methyl ester **149**.*

Synthesised in analogous manner to diol **29**. Thus methyl ester **149** (0.039 g, 0.073 mmol) in THF (6 cm^3) along with lithium aluminium hydride (0.50 cm^3, 1.0 M in THF, 0.50 mmol) gave after stirring for 18 hours diol **151** (0.029 g, 78%), as an oil, R_f [hexane:EtOAc (7:3)] 0.12; $[\alpha]_D$ -7.63 (*c* 0.76, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3419, 2931, 1611, 1587, 1512; δ_H (250 MHz; $CDCl_3$) 7.36–7.05 (15H, m, Ar*H*), 7.02 (2H, d, *J* 8.7, Ar*H*), 6.80 (2H, d, *J* 8.7, Ar*H*), 4.47 (1H, d, *J* 11.8, OCH_AH_BPh), 4.31 (1H, d, *J* 11.8, OCH_AH_BPh), 4.18 (1H, br t, *J* 5.5, C_3H), 3.82 (3H, s, O*Me*), 3.71 (2H, d, *J* 13.9, $NCH_XH_YPh \times 2$), 3.63 (2H, d, *J* 13.9, NCH_XH_YPh), 3.53 (2H, d, *J* 3.5, CH_2OH), 3.47 (1H, dt, *J* 6.0, 3.5, C_2H), 3.12 (1H, dt, *J* 7.6, 5.5, C_4H), 2.99 (1H, dd, *J* 14.2, 7.6, $C_5H_SH_T$), 2.87 (1H, dd, *J* 14.2, 5.5, $C_5H_SH_T$); δ_c (62.9 MHz; $CDCl_3$) 157.7 (1C, Q), 139.7 (2C, Q), 137.6 (1C, Q), 132.9 (1C, Q), 130.4 (2C, CH), 128.7 (4C, CH), 128.4 (2C, CH), 128.1 (4C, CH), 127.8 (2C, CH), 127.7 (1C, CH), 126.7 (2C, CH), 113.5 (2C, CH), 78.7 (1C, CH), 71.4 (1C, CH), 71.2 (1C, CH_2), 60.4 (1C, CH_2), 60.1 (1C, CH), 55.2 (1C, CH_3), 54.3 (2C, CH_2), 31.1 (1C, CH_2); *m/z* (FAB) 512 ($[M + H]^+$,

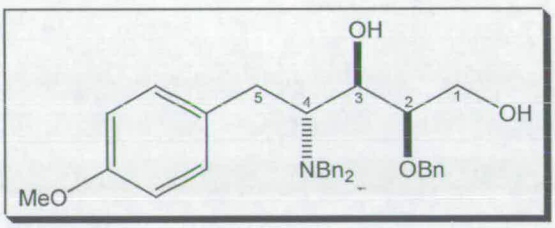
25%), 510 (34), 330 (41), 390 (24), 121 (19), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 512.2818. $C_{33}H_{38}NO_4$ requires *m/z* 512.2801).

*Via reduction of aldol adduct **156**.*

Synthesised in analogous manner to diol **29**. Thus, to aldol adduct **156** (0.123 g, 0.180 mmol) in THF (10 cm^3) was added methanol (0.36 cm^3, 0.028 g, 0.90 mmol) and lithium borohydride (0.020 g, 0.90 mmol). The resulting solution was stirred at room temperature for 18 hours to give the diol **151** (0.068 g, 75%) as an oil.

All spectroscopic data was identical to the compound synthesised above.

(2*R*,3*R*,4*R*)-2-Benzyloxy-4-*N*,*N*-dibenzylamino-5-(4-methoxyphenyl)pentane-1,3-diol 153



*Via reduction of methyl ester **148**.*

Analogous to the above protocol, thus methyl ester **148** (0.0982 g, 0.182 mmol) in THF (8 cm^3) along with lithium aluminium hydride (0.55 cm^3, 1.0 M in THF, 0.55 mmol) gave after stirring for 18 hours diol **153** (0.0619 g, 67%), as an oil, R_f [hexane:EtOAc (7:3)] 0.21; $[\alpha]_D$ -34.0 (*c* 1.68, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3444, 3031, 2933, 1611, 1511; δ_H (600 MHz; $CDCl_3$) 7.26–6.98 (15H, m, Ar*H*), 7.02 (2H, d, *J* 8.1, Ar*H*), 6.72 (2H, d, *J* 8.1, Ar*H*), 4.20 (1H, d, *J* 11.2, OCH_AH_BPh), 3.80 (1H, br s, C_3H), 3.77 (1H, d, *J* 11.2, OCH_AH_BPh), 3.72 (3H, s, O*Me*), 3.66 (2H, d, *J* 13.7, NCH_XH_YPh), 3.55 (2H, d, *J* 13.7, NCH_XH_YPh), 3.55–3.51 (1H, m, $C_1H_SH_T$), 3.41 (1H, dd, *J* 11.7, 4.2, $C_1H_SH_T$), 3.35 (1H, q, *J* 4.2, C_2H), 3.02 (1H, q, *J* 6.3, C_4H), 2.95

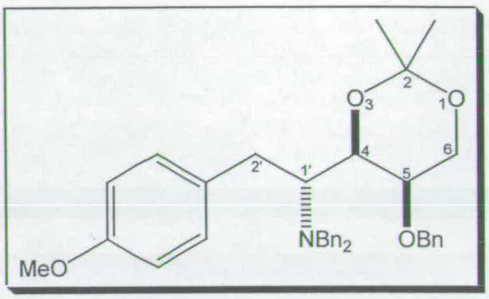
(1H, dd, *J* 14.1, 6.2, $C_5H_MH_N$), 2.84 (1H, dd, *J* 14.1, 6.8, $C_5H_MH_NAr$), 2.31 (1H, br s, O*H*), 2.89 (1H, br s, O*H*); δ_c (62.9 MHz; $CDCl_3$) 158.2 (1C, Q), 140.5 (2C, Q), 138.4 (1C, Q), 133.8 (1C, Q), 130.8 (2C, CH), 129.5 (4C, CH), 128.8 (2C, CH), 128.7 (4C, CH), 128.2 (1C, CH), 128.1 (2C, CH), 127.4 (2C, CH), 114.1 (2C, CH), 79.3 (1C, CH), 73.0 (1C, CH_2), 72.4 (1C, CH), 62.6 (1C, CH_2), 61.1 (1C, CH), 55.7 (1C, CH_3), 54.9 (2C, CH_2), 31.3 (1C, CH_2); m/*z* (FAB) 512 ($[M + H]^+$, 65%), 390 (13), 240 (9), 121 (19), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 512.2801. $C_{33}H_{38}NO_4$ requires *m/z* 512.2801).

Via reduction of aldol adduct ***155****.*

Synthesised in analogous manner to diol **29**. Thus, to a solution of crude aldol adduct **155** (1.13 g) in THF (20 cm^3) was added methanol (1.0 cm^3, 0.79 g, 25 mmol) and lithium borohydride (0.55 g, 25 mmol). The resulting solution was stirred at room temperature for 18 hours to give the diol **153** (0.151 g) as an oil.

All spectroscopic data was identical to the compound synthesised above.

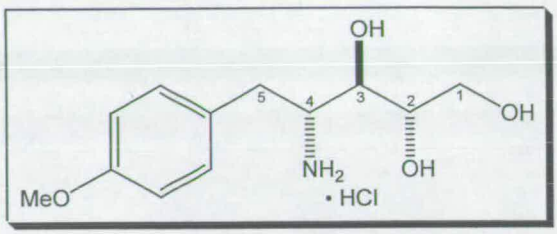
(1'*R*,4*R*,5*R*)-5-Benzyloxy-2,2-trimethyl-4-[1'-*N*,*N*-dibenzylamino-2'-(4-methoxyphenyl) ethyl]-1,3-dioxane 154



To a solution of diol **153** (0.0597 g, 0.117 mmol) in acetone (50 cm^3) was added a few mg of iodine. The resulting solution was heated to reflux and held there for 3 hours before being cooled to room temperature. 1 M aqueous sodium hydroxide (≈1 cm^3) was added until the solution lost its brown appearance, and the organic phase was separated. The aqueous phase was extracted with $CHCl_3$ (3 × 15 cm^3) and the combined organic phase was washed with water (50 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (10:1)] to give **154** (0.0195 g, 30%) as an oil, R_f [hexane:EtOAc (7:3)] 0.75; $[\alpha]_D$ -36.0 (*c* 0.30, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3061, 2935, 1611, 1584, 1512; δ_H (360 MHz; $CDCl_3$) 7.19–6.99 (15H, m, Ar*H*), 6.93 (2H, d, *J* 8.7, Ar*H*), 6.69 (2H, d, *J* 8.7, Ar*H*), 4.41 (1H, d, *J* 12.0, OCH_AH_BPh), 4.17 (1H, d, *J* 12.0, OCH_AH_BPh), 4.04 (1H, dd, *J* 4.0, 2.6, C_4H), 3.86 (1H, dd, *J* 12.7, 2.6, $C_6H_SH_T$), 3.76 (3H, s, O*Me*), 3.75 (1H, dd, *J* 12.7, 2.6, $C_6H_SH_T$), 3.71 (2H, d, *J* 14.0, NCH_XH_YPh), 3.40 (2H, d, *J* 14.0, NCH_XH_YPh), 3.26 (1H, dt, 10.0, 3.8, $C_1{\cdot}H$), 3.23 (1H, q, *J* 2.6, C_5H), 3.03 (1H, dd, *J* 14.8, 3.6, $C_{2'}H_MH_N$), 2.79 (1H, dd, *J* 14.8, 10.0, $C_{2'}H_MH_N$), 1.38 (3H, s, C_2Me), 1.35 (3H, s, C_2Me); δ_c (62.9 MHz; $CDCl_3$) 157.4 (1C, Q), 140.0 (2C, Q), 138.1 (1C, Q), 133.9 (1C, Q), 130.5 (2C, CH), 128.6 (4C, CH), 128.2 (2C, CH), 128.0 (4C, CH), 127.7 (2C, CH), 127.4 (1C, CH), 126.6 (2C, CH), 113.1 (2C, CH), 98.8 (1C, Q), 72.8 (1C, CH), 71.1 (1C, CH_2), 69.8 (1C, CH), 61.6 (1C, CH_2), 60.4 (1C, CH), 55.2 (1C, CH_3), 54.5 (2C, CH_2), 32.0 (1C, CH_2), 28.6 (1C, CH_3), 19.3 (1C, CH_3); *m/z* (FAB) 552 ($[M + H]^+$, 19%), 550 (27), 430 (24), 330 (28), 121 (23),

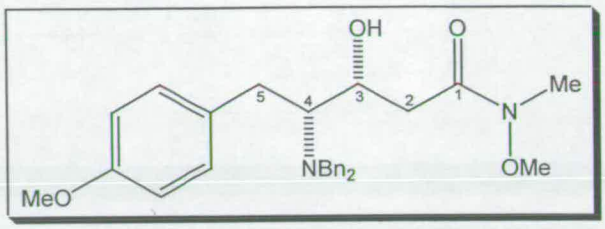
91 (100); HRMS (FAB) (Found: $[M + H]^+$, 552.3114. $C_{36}H_{42}NO_4$ requires *m/z* 552.3116).

(2*S*,3*R*,4*R*)-4-Amino-2,3-dihydroxy-5-(4-methoxyphenyl)pentan-1-ol hydrochloride 152



To a solution of the diol **151** (0.0213 g, 0.0417 mmol) and Pearlman's catalyst [0.0213 g; 20% $Pd(OH)_2/C$] in methanol (3 cm^3) was added hydrochloric acid (0.084 cm^3, 1 M in ether, 0.084 mmol). The solution was exposed to a hydrogen atmosphere (1 atm) and stirred vigorously for 18 hours. The mixture was then filtered through a pad of Celite and concentrated under reduced pressure to give the hydrochloride salt **152** (0.0116 g, 100%) as a white solid, mp 197–199 °C; $[\alpha]_D$ +45.3 (*c* 0.23, CH_3OH); ν_{max} (KBr)/cm^{-1} 3322, 3009, 2933, 1610, 1576, 1513; δ_H (250 MHz; CD_3OD) 7.32 (2H, d, *J* 8.7, Ar*H*), 7.02 (2H, d, *J* 8.7, Ar*H*), 3.93–3.86 (1H, m, C_2H), 3.91 (1H, dd, *J* 8.9, 5.0, C_3H), 3.88 (3H, s, O*Me*), 3.81–3.79 (1H, m, C_4H), 3.76 (1H, dd, *J* 7.8, 3.1, $C_1H_AH_B$), 3.71 (1H, dd, *J* 7.8, 2.5, $C_1H_AH_B$), 3.25 (1H, dd, *J* 14.5, 3.7, $C_5H_XH_Y$), 2.85 (1H, dd, *J* 14.5, 10.8, $C_5H_XH_Y$); δ_c (62.9 MHz; CD_3OD) 158.6 (1C, Q), 129.6 (2C, CH), 127.3 (1C, Q), 113.6 (2C, CH), 71.4 (1C, CH), 69.5 (1C, CH), 62.7 (1C, CH_2), 54.9 (1C, CH), 53.8 (1C, CH_3), 31.0 (1C, CH_2); m/z (FAB) 242 ($[M + H]^+$, 99%), 150 (29), 135 (28), 121 (100), 91 (32); HRMS (FAB) (Found: $[M + H]^+$, 242.1397. $[C_{12}H_{20}NO_4]^+Cl^-$ requires *m/z* 242.1392).

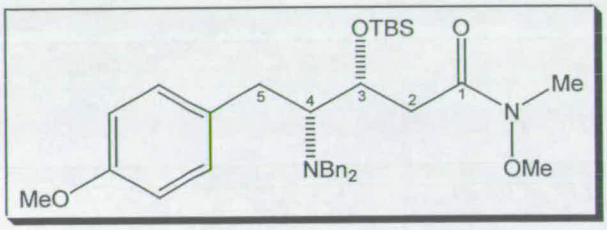
(3*RS*,4*RS*)-4-*N*,*N*-Dibenzylamino-3-hydroxy-5-(4-methoxyphenyl)pentanoic acid methoxy methyl amide 190



To a suspension of *N*,*O*-dimethylhydroxylamine·hydrochloride (0.763 g, 7.83 mmol) in THF (5 cm^3) at 0 °C was added trimethylaluminium (3.9 cm^3, 2.0 M in toluene, 7.8 mmol). The clear solution produced was allowed to stir for 30 minutes before being added to a solution of ethyl ester **98** (0.500 g, 1.11 mmol) in THF (15 cm^3) at 0 °C *via* cannula. The mixture produced was warmed to room temperature and stirred for 4 hours, before being quenched by the addition of saturated aq. sodium potassium tartrate (50 cm^3). The biphasic mixture was stirred vigorously for 15 hours by which time two clear phases were apparent. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 25 cm^3). The combined organics were dried, and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [hexane:EtOAc (4:1)] to give **190** (0.503 g, 97%) as a white solid, R_f [hexane:EtOAc (7:3)] 0.12; mp 123–125 °C; ν_{max} (KBr)/cm^{-1} 3457, 3026, 2934, 1643, 1613, 1584, 1512; δ_H (250 MHz; $CDCl_3$) 7.40–7.29 (10H, m, Ar*H*), 7.21 (2H, d, *J* 8.6, Ar*H*), 6.90 (2H, d, *J* 8.6, Ar*H*), 4.23 (2H, d, *J* 13.5, NC$H_X$$H_Y$Ph × 2), 4.04 (1H, ddd, *J* 10.2, 5.2, 2.0, C_3H), 3.85 (3H, s, ArO*Me*), 3.60 (3H, s, NO*Me*), 3.52 (2H, d, *J* 13.5, NC$H_X$$H_Y$Ph × 2), 3.15 (1H, dd, *J* 13.4, 4.5, $C_5H_M$$H_N$), 3.18 (3H, s, N*Me*), 3.01 (1H, dd, *J* 13.4, 9.4, $C_5H_M$$H_N$), 2.89 (1H, dd, *J* 16.7, 10.2, $C_2H_S$$H_T$), 2.71 (1H, dt, *J* 9.4, 4.8, C_4H), 2.11 (1H, br d, *J* 16.7, $C_2H_S$$H_T$); δ_c (62.9 MHz; $CDCl_3$) 174.7 (1C, Q), 158.4 (1C, Q), 140.5 (2C, Q), 132.8 (1C, Q), 130.7 (2C, CH), 129.5 (4C, CH), 128.7 (4C, CH), 127.4 (2C, CH), 114.4 (2C, CH), 68.4 (1C, CH), 63.7 (1C, CH), 61.6 (1C, CH_3), 55.7 (1C, CH_3), 55.4 (2C, CH_2), 37.2 (1C, CH_2), 32.3 (1C, CH_3), 29.9 (1C, CH_2); m/*z* (FAB) 463 ($[M + H]^+$, 22%), 330

(24), 121 (22), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 463.2591. $C_{28}H_{35}N_2O_4$ requires *m/z* 463.2597).

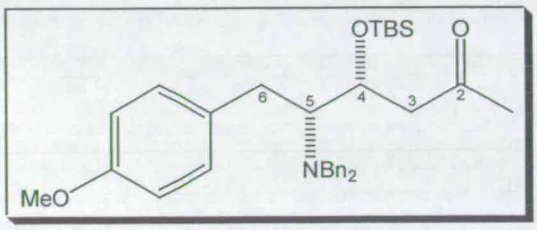
(3*RS*,4*RS*)-3-*tert*-Butyldimethylsilyloxy-4-*N*,*N*-dibenzylamino-5-(4-methoxyphenyl)pentanoic acid methoxy methyl amide 189



To a solution of alcohol **190** (0.381 g, 0.827 mmol) in CH_2Cl_2 (5 cm^3) was added 2,6-lutidine (0.57 cm^3, 0.53 g, 4.9 mmol), and *tert*-butyldimethylsilyl triflate (0.57 cm^3, 0.53 g, 4.9 mmol). The solution was stirred at room temperature for 4 hours before being quenched by the addition of water (20 cm^3). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 cm^3); the combined organic phase was washed with brine (30 cm^3) then dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (4:1)] to give **189** (0.397 g, 84%) as a white solid, R_f [hexane:EtOAc (7:3)] 0.31; mp 108–109 °C; ν_{max} (KBr)/cm^{-1} 3027, 2925, 2800, 1666, 1611, 1581, 1511; δ_H (250 MHz; $CDCl_3$) 7.30–7.18 (10H, m, Ar*H*), 7.22 (2H, d, *J* 8.6, ArH), 6.91 (2H, d, *J* 8.6, Ar*H*), 4.31 (1H, ddd, *J* 6.7, 5.0, 2.1, C_3H), 4.15 (2H, d, *J* 13.4, NCH_XH_YPh × 2), 3.84 (3H, s, ArO*Me*), 3.66 (3H, s, NO*Me*), 3.46 (2H, d, *J* 13.4, NCH_XH_YPh × 2), 3.18–2.90 (2H, m, $C_2H_SH_T$ + C_4H), 3.14 (1H, dd, *J* 12.1, 5.6, $C_5H_AH_B$), 3.07 (3H, s, N*Me*), 2.96 (1H, dd, *J* 12.1, 6.4, $C_5H_AH_B$), 2.59 (1H, dd, *J* 16.9, 5.0, $C_2H_SH_T$), 0.86 (9H, s, ^tBu), 0.00 (3H, s, $SiCH_3$), -0.02 (3H, s, $SiCH_3$); δ_c (62.9 MHz; $CDCl_3$) 177.5 (1C, Q), 158.3 (1C, Q), 141.2 (2C, Q), 133.1 (1C, Q), 130.7 (2C, CH), 129.6 (4C, CH), 128.5 (4C, CH), 127.1 (2C, CH), 114.3 (2C, CH), 71.7 (1C, CH), 62.6 (1C, CH), 61.5 (1C, CH_3), 56.1 (2C, CH_2), 55.7 (1C, CH_3), 37.8

(1C, CH_2), 32.4 (1C, CH_3), 31.1 (1C, CH_2), 26.5 (3C, CH_3), 18.7 (1C, Q), -3.9 (1C, CH_3), -4.5 (1C, CH_3); m/z (FAB) 577 ($[M + H]^+$, 49%), 455 (50), 330 (100), 121 (67), 91 (100); HRMS (FAB) (Found: $[M + H]^+$, 577.3455. $C_{34}H_{49}N_2O_4Si$ requires *m/z* 577.3462).

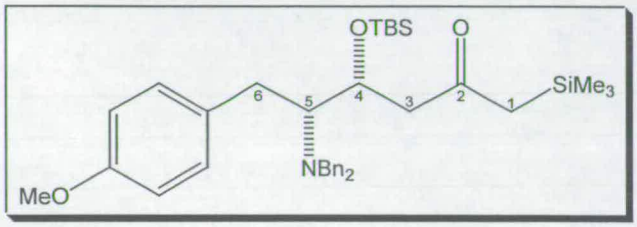
(4*RS*,5*RS*)-4-*tert*-Butyldimethylsilyloxy-5-*N*,*N*-dibenzylamino-6-(4-methoxyphenyl)hexan-2-one 191



To a solution of Weinreb amide **189** (0.0750 g, 0.13 mmol) in THF (5 cm^3) at -45 °C was added (trimethylsilyl)methyllithium (0.45 cm^3, 1.0 M in pentane, 0.45 mmol). The mixture produced was stirred for 2 hours before being quenched by the addition of 2 M anhydrous acetic acid (1 cm^3) and pH 7 phosphate buffer (10 cm^3). The organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 20 cm^3). The combined organics were dried, and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [hexane:EtOAc (9:1)] to give **191** (0.0623 g, 90%) as an oil, R_f [hexane:EtOAc (7:3)] 0.61; ν_{max} (neat)/cm^{-1} 3061, 2929, 1716, 1613, 1583, 1512; δ_H (250 MHz; $CDCl_3$) 7.38–7.23 (10H, m, Ar*H*), 7.26 (2H, d, *J* 8.7, Ar*H*), 6.95 (2H, d, *J* 8.7, Ar*H*), 4.20 (1H, ddd, *J* 7.5, 4.1, 1.8, C_4H), 4.16 (2H, d, *J* 13.4, NCH_AH_BPh × 2), 3.88 (3H, s, O*Me*), 3.42 (2H, d, *J* 13.4, NCH_AH_BPh × 2), 3.20 (1H, br t, *J* 8.2, C_5H), 3.11 (1H, dd, *J* 18.2, 7.4, $C_3H_SH_T$), 3.01–2.91 (2H, m, $C_6H_XH_Y + C_6H_XH_Y$), 2.53 (1H, dd, *J* 18.2, 4.1, $C_3H_SH_T$), 1.78 (3H, s, $COCH_3$), 0.88 (9H, s, *t-Bu*), 0.02 (3H, s, $SiCH_3$), -0.01 (3H, s, $SiCH_3$); δ_c (62.9 MHz; $CDCl_3$) 207.3 (1C, Q), 158.3 (1C, Q), 141.3 (2C, Q), 132.8 (1C, Q), 130.6 (2C, CH), 129.7 (4C, CH), 128.6 (4C, CH), 127.2 (2C, CH), 114.3 (2C, CH),

70.6 (1C, CH), 61.3 (1C, CH), 56.4 (2C, CH_2), 55.7 (1C, CH_3), 48.8 (1C, CH_2), 31.0 (1C, CH_2), 30.8 (1C, CH_3), 26.3 (3C, CH_3), 18.5 (1C, Q), -4.0 (1C, CH_3), -4.4 (1C, CH_3); *m/z* (FAB) 532 ($[M + H]^+$, 23%), 530 (25), 410 (81), 330 (100), 121 (76), 91 (98); HRMS (FAB) (Found: $[M + H]^+$, 532.3252. $C_{33}H_{46}NO_3Si$ requires *m/z* 532.3247).

(4*RS*,5*RS*)-4-*tert*-Butyldimethylsilyloxy-5-*N*,*N*-dibenzylamino-6-(4-methoxyphenyl)-1-trimethylsilylhexan-2-one 188



To a solution of Weinreb amide **189** (0.0656 g, 0.113 mmol) in THF (5 cm^3) at -45 °C was added (trimethylsilyl)methyllithium (0.41 cm^3, 1.0 M in pentane, 0.41 mmol). The mixture produced was stirred for 2 hours before being quenched by the addition of pH 7 phosphate buffer (10 cm^3). The organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 20 cm^3). The combined organics were dried, and concentrated under reduced pressure to give **188** (0.0690 g, 100%) as an oil, which was not purified further. R_f [hexane:EtOAc (4:1)] 0.78; δ_H (200 MHz; $CDCl_3$) 7.30–7.23 (12H, m, Ar*H*), 6.94 (2H, d, *J* 8.6, Ar*H*), 4.27–4.18 (1H, m, C_4H), 4.15 (2H, d, *J* 13.3, NCH_XH_YPh × 2), 3.87 (3H, s, O*Me*), 3.45 (2H, d, *J* 13.3, NCH_XH_YPh), 3.16–2.83 (4H, $C_6H_AH_B + C_6H_AH_B + C_5H + C_3H_MH_N$), 2.59 (1H, dd, *J* 17.3, 5.3, $C_3H_MH_N$), 1.99 (1H, d, *J* 10.5, $C_1H_SH_T$), 1.87 (1H, d, *J* 10.5, $C_1H_SH_T$), 0.88 (9H, s, ^tBu), 0.10 (9H, s, SiMe_3), 0.03 (3H, s, SiCH_3), 0.00 (3H, s, SiCH_3).

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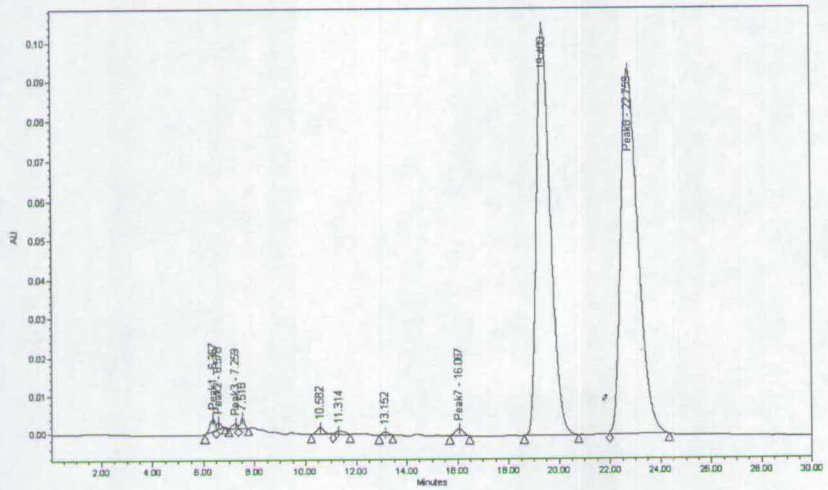
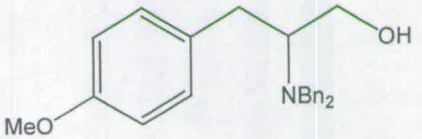
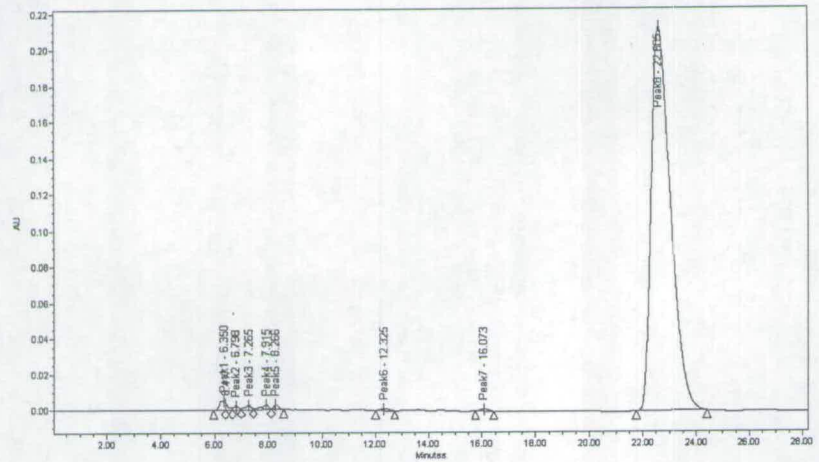
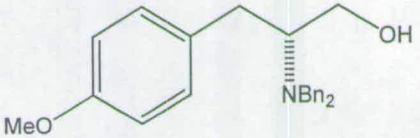
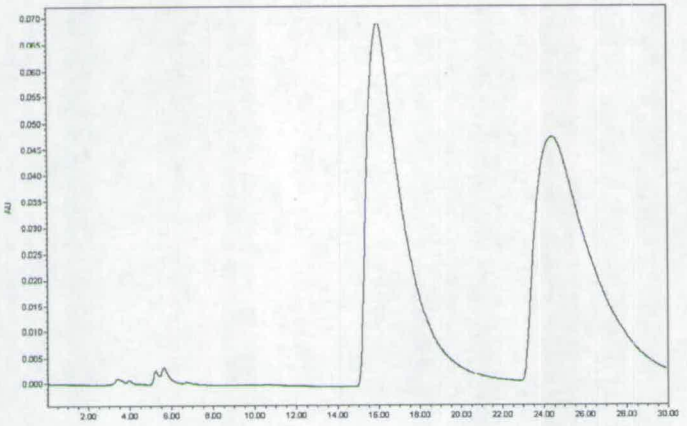


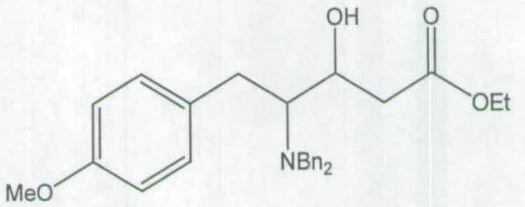


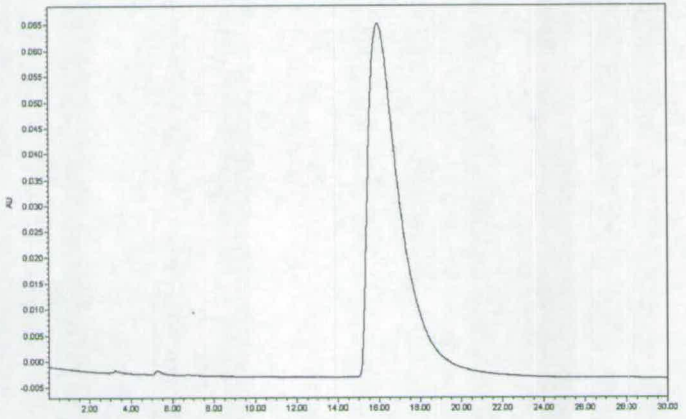


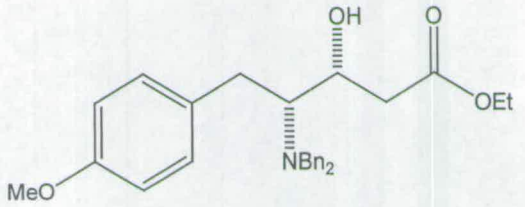


Chiral HPLC Traces For Amino Alcohol 42

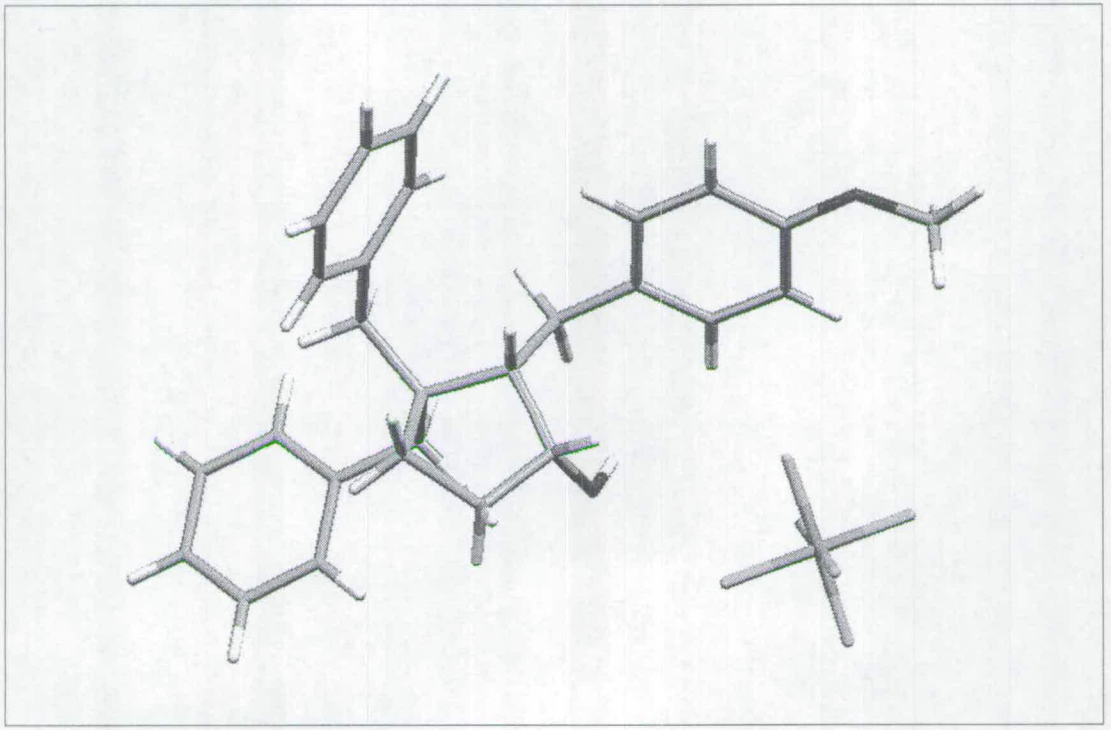








Chiral HPLC Traces For β-hydroxy ester 98



X-ray Crystal Structure of 110

Table 1: *Crystal data and structure refinement for* ***110***

Part A: CRYSTAL DATA	
Empirical formula	$C_{26.50}H_{32}F_6NO_{2.50}P$
Formula weight	549.50
Wavelength	0.71073 Å
Temperature	150(2) K
Crystal system	Monoclinic
Space group	C2
Unit cell dimensions	a = 19.914(2) Å alpha = 90 deg.
	b = 9.8326(11) Å beta = 111.621(2) deg.
	c = 13.9100(16) Å gamma = 90 deg.
Volume	2532.0(5) $Å^3$
Number of reflections for cell	4534 (2 < theta < 27 deg.)
Z	4
Density (calculated)	1.441 Mg/m^3
Absorption coefficient	0.181 mm^{-1}
F(000)	1148
Part B: DATA COLLECTION	
Crystal description	Colourless block
Crystal size	0.54 x 0.18 x 0.18 mm
Instrument	CCD area detector
Theta range for data collection	1.57 to 29.04 deg.
Index ranges	-26<=h<=25, -13<=k<=13, -18<=l<=18
Reflections collected	11621
Independent reflections	6012 [R(int) = 0.0243]
Scan type	Phi and omega scans
Absorption correction	Multiscan (Tmin= 0.907, Tmax=1)

Part C: SOLUTION AND REFINEMENT	
Solution	Direct (SHELXS-97 (Sheldrick, 1990))
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	Geom
Hydrogen atom treatment	Riding, rotating rigid group or restrained refall
Data / restraints / parameters	6012/3/351
Goodness-of-fit on F^2	1.044
Conventional R [F>4sigma(F)]	R1 = 0.0404 [5211 data]
Weighted R (F^2 and all data)	wR2 = 0.0972
Absolute structure parameter	-0.05(8) -Strong enantiodistinguishing power
Final maximum delta/sigma	0.003
Weighting scheme	calc w=1/[\s^2^(Fo^2^)+(0.0529P)^2^+0.0000 P] where P=(Fo^2^+2Fc^2^)/3
Largest diff. peak and hole	0.267 and -0.244 e. $Å^{-3}$

Table 2: *Atomic coordinates (× 10^4), equivalent isotropic displacement parameters ($Å^2$ × 10^3) and site occupancies for* ***110****. U(eq)is defined as one third of the trace of the orthogonalized Uij tensor.*

	x	y	z	U(eq)	Occ
N(1)	491(1)	1710(2)	-2179(1)	24(1)	1
C(2)	884(1)	546(2)	-1455(1)	24(1)	1
O(3)	976(1)	1340(2)	258(1)	36(1)	1
C(3)	1345(1)	1269(2)	-448(1)	27(1)	1
C(4)	1483(1)	2699(2)	-791(2)	30(1)	1
C(5)	1094(1)	2737(2)	-1963(1)	28(1)	1
C(10)	174(1)	1316(2)	-3324(1)	29(1)	1
C(11)	680(1)	581(2)	-3725(1)	31(1)	1
C(12)	666(1)	-827(2)	-3796(2)	36(1)	1
C(13)	1117(1)	-1511(3)	-4195(2)	45(1)	1
C(14)	1573(1)	-781(3)	-4545(2)	45(1)	1
C(15)	1588(1)	609(3)	-4489(2)	44(1)	1
C(16)	1139(1)	1300(2)	-4091(2)	36(1)	1
C(20)	-129(1)	2248(2)	-1868(1)	27(1)	1
C(21)	-482(1)	3526(2)	-2416(1)	27(1)	1
C(22)	-1088(1)	3454(2)	-3324(2)	33(1)	1
C(23)	-1421(1)	4635(2)	-3822(2)	40(1)	1
C(24)	-1163(1)	5885(2)	-3412(2)	42(1)	1
C(25)	-568(1)	5968(2)	-2497(2)	40(1)	1
C(26)	-233(1)	4797(2)	-2005(2)	33(1)	1
C(30)	408(1)	-598(2)	-1343(2)	30(1)	1
C(31)	874(1)	-1789(2)	-782(2)	28(1)	1
C(32)	974(1)	-2066(2)	235(2)	34(1)	1
C(33)	1417(1)	-3113(2)	781(2)	32(1)	1
C(34)	1777(1)	-3908(2)	304(1)	26(1)	1
C(35)	1681(1)	-3657(2)	-725(1)	28(1)	1
C(36)	1237(1)	-2604(2)	-1248(1)	28(1)	1
O(37)	2248(1)	-4924(2)	788(1)	31(1)	1
C(38)	2269(1)	-5342(2)	1782(2)	39(1)	1
P(1)	2397(1)	29(1)	2940(1)	36(1)	1
F(1)	2218(1)	1360(2)	2237(1)	83(1)	1
F(2)	2557(1)	-1293(2)	3633(1)	77(1)	1
F(3)	3234(1)	285(2)	3251(1)	81(1)	1
F(4)	1556(1)	-236(2)	2610(1)	81(1)	1
F(5)	2411(1)	924(2)	3899(1)	60(1)	1
F(6)	2404(1)	-821(2)	1980(1)	70(1)	1
C(1M)	5000	199(5)	5000	74(1)	1
O(1M)	4650(2)	-870(5)	4624(5)	100(2)	0.50

Table 3: *Bond lengths for* ***110***

Bond	Length Å
N(1)-C(5)	1.513(2)
N(1)-C(10)	1.531(2)
N(1)-C(2)	1.535(2)
N(1)-C(20)	1.544(2)
C(2)-C(30)	1.515(3)
C(2)-C(3)	1.537(2)
O(3)-C(3)	1.429(2)
C(3)-C(4)	1.542(3)
C(4)-C(5)	1.526(3)
C(10)-C(11)	1.505(3)
C(11)-C(12)	1.387(3)
C(11)-C(16)	1.391(3)
C(12)-C(13)	1.390(3)
C(13)-C(14)	1.380(4)
C(14)-C(15)	1.368(3)
C(15)-C(16)	1.392(3)
C(20)-C(21)	1.503(2)
C(21)-C(26)	1.388(3)
C(21)-C(22)	1.390(3)
C(22)-C(23)	1.389(3)
C(23)-C(24)	1.372(3)
C(24)-C(25)	1.386(3)
C(25)-C(26)	1.380(3)
C(30)-C(31)	1.519(3)
C(31)-C(32)	1.382(3)
C(31)-C(36)	1.389(3)
C(32)-C(33)	1.386(3)
C(33)-C(34)	1.383(3)
C(34)-O(37)	1.366(2)
C(34)-C(35)	1.394(3)
C(35)-C(36)	1.382(3)
O(37)-C(38)	1.428(2)
P(1)-F(2)	1.5790(16)
P(1)-F(3)	1.5805(15)
P(1)-F(6)	1.5808(15)
P(1)-F(4)	1.5855(15)
P(1)-F(5)	1.5888(14)
P(1)-F(1)	1.5943(16)
C(1M)-O(1M)	1.264(5)

Table 4: *Bond angles for* ***110.***

Bond	Angle (degrees)
C(5)-N(1)-C(10)	111.58(14)
C(5)-N(1)-C(2)	101.05(12)
C(10)-N(1)-C(2)	114.16(13)
C(5)-N(1)-C(20)	111.93(14)
C(10)-N(1)-C(20)	108.22(13)
C(2)-N(1)-C(20)	109.85(12)
C(30)-C(2)-N(1)	115.70(14)
C(30)-C(2)-C(3)	115.84(15)
N(1)-C(2)-C(3)	104.01(14)
O(3)-C(3)-C(2)	111.28(15)
O(3)-C(3)-C(4)	111.26(16)
C(2)-C(3)-C(4)	104.76(14)
C(5)-C(4)-C(3)	105.94(15)
N(1)-C(5)-C(4)	104.43(14)
C(11)-C(10)-N(1)	115.59(14)
C(12)-C(11)-C(16)	118.7(2)
C(12)-C(11)-C(10)	120.5(2)
C(16)-C(11)-C(10)	120.72(19)
C(11)-C(12)-C(13)	120.8(2)
C(14)-C(13)-C(12)	119.7(2)
C(15)-C(14)-C(13)	120.2(2)
C(14)-C(15)-C(16)	120.4(2)
C(11)-C(16)-C(15)	120.2(2)
C(21)-C(20)-N(1)	114.63(14)
C(26)-C(21)-C(22)	118.65(18)
C(26)-C(21)-C(20)	120.93(16)
C(22)-C(21)-C(20)	120.35(18)
C(23)-C(22)-C(21)	120.4(2)
C(24)-C(23)-C(22)	120.29(18)
C(23)-C(24)-C(25)	119.8(2)
C(26)-C(25)-C(24)	120.0(2)
C(25)-C(26)-C(21)	120.82(18)
C(2)-C(30)-C(31)	109.75(14)
C(32)-C(31)-C(36)	117.46(17)
C(32)-C(31)-C(30)	120.50(17)
C(36)-C(31)-C(30)	122.00(16)
C(31)-C(32)-C(33)	121.99(18)
C(34)-C(33)-C(32)	119.62(17)
O(37)-C(34)-C(33)	124.06(16)
O(37)-C(34)-C(35)	116.34(16)
C(33)-C(34)-C(35)	119.57(17)
C(36)-C(35)-C(34)	119.49(17)

C(35)-C(36)-C(31)	121.87(17)
C(34)-O(37)-C(38)	117.17(15)
F(2)-P(1)-F(3)	90.53(11)
F(2)-P(1)-F(6)	91.05(10)
F(3)-P(1)-F(6)	88.74(9)
F(2)-P(1)-F(4)	89.75(11)
F(3)-P(1)-F(4)	178.99(10)
F(6)-P(1)-F(4)	90.29(10)
F(2)-P(1)-F(5)	90.34(9)
F(3)-P(1)-F(5)	89.52(9)
F(6)-P(1)-F(5)	177.78(10)
F(4)-P(1)-F(5)	91.45(9)
F(2)-P(1)-F(1)	178.77(12)
F(3)-P(1)-F(1)	90.69(12)
F(6)-P(1)-F(1)	89.12(10)
F(4)-P(1)-F(1)	89.04(11)
F(5)-P(1)-F(1)	89.52(9)

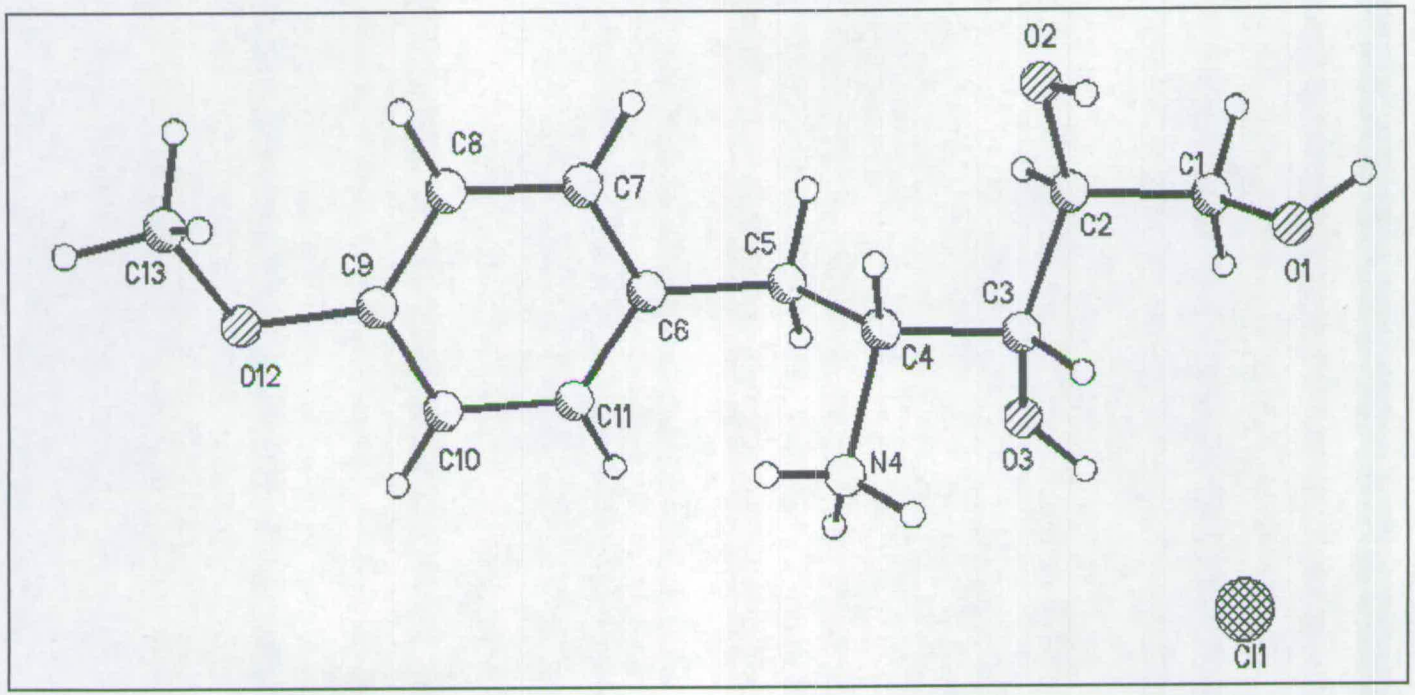
Symmetry transformations used to generate equivalent atoms:

Table 5: *Anisotropic displacement parameters ($Å^2$ x 10^3) for **110**. The anisotropic displacement factor exponent takes the form: -2 pi^2 [h^2 a*^U11 + ... + 2 h k a* b* U12]*

	U11	U22	U33	U23	U13	U12
N(1)	19(1)	26(1)	24(1)	1(1)	8(1)	-1(1)
C(2)	21(1)	25(1)	26(1)	1(1)	7(1)	2(1)
C(3)	22(1)	35(1)	25(1)	1(1)	8(1)	4(1)
C(4)	24(1)	31(1)	32(1)	-4(1)	8(1)	-3(1)
C(5)	24(1)	26(1)	34(1)	1(1)	11(1)	-3(1)
C(10)	25(1)	35(1)	23(1)	2(1)	6(1)	0(1)
C(11)	26(1)	40(1)	20(1)	-4(1)	2(1)	2(1)
C(12)	33(1)	42(1)	28(1)	-7(1)	7(1)	-1(1)
C(13)	43(1)	47(1)	36(1)	-15(1)	2(1)	7(1)
C(14)	34(1)	66(2)	33(1)	-13(1)	9(1)	10(1)
C(15)	38(1)	65(2)	33(1)	-2(1)	16(1)	4(1)
C(16)	36(1)	43(1)	29(1)	1(1)	14(1)	2(1)
C(20)	22(1)	30(1)	31(1)	4(1)	13(1)	4(1)
C(21)	23(1)	31(1)	28(1)	2(1)	11(1)	3(1)
C(22)	26(1)	38(1)	32(1)	-3(1)	8(1)	1(1)
C(23)	29(1)	56(1)	32(1)	8(1)	7(1)	11(1)
C(24)	39(1)	41(1)	51(1)	20(1)	23(1)	16(1)
C(25)	36(1)	30(1)	59(1)	0(1)	22(1)	1(1)
C(26)	23(1)	37(1)	37(1)	-4(1)	9(1)	1(1)
C(30)	23(1)	29(1)	37(1)	4(1)	13(1)	2(1)
C(31)	22(1)	26(1)	36(1)	5(1)	11(1)	-2(1)
C(32)	36(1)	34(1)	39(1)	2(1)	23(1)	6(1)
C(33)	35(1)	34(1)	31(1)	4(1)	17(1)	3(1)
C(34)	19(1)	28(1)	28(1)	0(1)	5(1)	-3(1)
C(35)	26(1)	28(1)	30(1)	-3(1)	12(1)	-1(1)
C(36)	25(1)	31(1)	28(1)	3(1)	9(1)	-3(1)
O(37)	29(1)	32(1)	30(1)	4(1)	10(1)	5(1)
C(38)	36(1)	44(1)	35(1)	13(1)	12(1)	7(1)
P(1)	35(1)	44(1)	29(1)	3(1)	12(1)	6(1)
F(1)	135(2)	63(1)	48(1)	21(1)	28(1)	3(1)
F(2)	104(1)	49(1)	77(1)	20(1)	32(1)	21(1)
F(3)	42(1)	110(2)	91(1)	-42(1)	25(1)	-18(1)
F(4)	36(1)	107(2)	97(1)	1(1)	22(1)	5(1)
F(5)	81(1)	66(1)	39(1)	1(1)	28(1)	23(1)
F(6)	74(1)	83(1)	61(1)	-33(1)	33(1)	-16(1)
C(1M)	95(3)	49(2)	105(3)	0	70(3)	0
O(1M)	63(3)	61(3)	185(6)	-47(3)	59(4)	-15(2)

Table 6: *Hydrogen coordinates (× 10^4) and isotropic displacement parameters ($Å^2$ × 10^3) for* ***110****.*

	x	y	z	U(eq)
H(2)	1226	137	-1750	29
H(3)	680(40)	710(70)	250(70)	33(8)
H(3')	1269(13)	1270(30)	864(15)	33(8)
H(3A)	1815	779	-118	33
H(4A)	1288	3406	-458	36
H(4B)	2007	2858	-601	36
H(5A)	898	3656	-2196	33
H(5B)	1425	2480	-2317	33
H(10A)	-254	733	-3440	34
H(10B)	5	2154	-3737	34
H(12)	345	-1329	-3569	43
H(13)	1110	-2476	-4227	55
H(14)	1878	-1244	-4825	54
H(15)	1908	1105	-4724	53
H(16)	1145	2266	-4068	43
H(20A)	-502	1531	-2009	32
H(20B)	63	2423	-1114	32
H(22)	-1275	2592	-3606	39
H(23)	-1830	4577	-4448	48
H(24)	-1391	6691	-3755	50
H(25)	-391	6832	-2208	48
H(26)	174	4862	-1376	40
H(30A)	75	-893	-2035	35
H(30B)	115	-275	-948	35
H(32)	732	-1522	571	40
H(33)	1474	-3284	1479	38
H(35)	1919	-4207	-1063	33
H(36)	1178	-2432	-1947	34
H(38A)	2451	-4594	2276	58
H(38B)	2589	-6131	2016	58
H(38C)	1781	-5588	1736	58
H(1M1)	4870	519	5576	110
H(1M2)	4883	904	4465	110
H(1M3)	5519	4	5251	110
H(1M)	4205	-702	4408	149



X-ray Crystal Structure of 152

Table 1: *Crystal data and structure refinement for* ***152***

Part A: CRYSTAL DATA	
Empirical formula	$C_{12}H_{20}ClNO_4$
Formula weight	277.74
Wavelength	0.71073 Å
Temperature	150(2) K
Crystal system	Orthorhombic
Space group	P2(1)2(1)2(1)
Unit cell dimensions	a = 5.5704(17) Å alpha = 90 deg.
	b = 8.711(3) Å beta = 90 deg.
	c = 28.470(9) Å gamma = 90 deg.
Volume	1381.5(7) $Å^3$
Number of reflections for cell	4146 (2 < theta < 28.5 deg.)
Z	4
Density (calculated)	1.335 Mg/m^3
Absorption coefficient	0.283 mm^{-1}
F(000)	592
Part B: DATA COLLECTION	
Crystal description	Colourless block
Crystal size	0.36 x 0.32 x 0.27 mm
Instrument	CCD area detector
Theta range for data collection	1.43 to 28.85 deg.
Index ranges	-7<=h<=7, -11<=k<=11, -38<=l<=37
Reflections collected	12119
Independent reflections	3385 [R(int) = 0.0359]
Scan type	Phi and omega scans
Absorption correction	Sadabs(Tmin= 0.849, Tmax=1)

Part C: SOLUTION AND REFINEMENT	
Solution	Patterson (DIRDIF)
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	Geometric/difmap (OH,Me,NH_3)
Hydrogen atom treatment	Riding/rotating group
Data / restraints / parameters	3385/0/169
Goodness-of-fit on F^2	1.154
Conventional R [F>4sigma(F)]	R1 = 0.0414 [3305 data]
Weighted R (F^2 and all data)	wR2 = 0.1043
Absolute structure parameter	-0.02(6)
Final maximum delta/sigma	0.036
Weighting scheme	calc w=1/[\s^2^(Fo^2^)+(0.0559P)^2^+0.3169P] where P=(Fo^2^+2Fc^2^)/3
Largest diff. peak and hole	0.337 and -0.274 e. $Å^{-3}$

Table 2: *Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters ($Å^2$ × 10^3) for* ***152****. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.*

	x	y	z	U(eq)
Cl(1)	8405(1)	8130(1)	7902(1)	29(1)
O(1)	3490(3)	9842(2)	7118(1)	32(1)
C(1)	5158(4)	9969(2)	6737(1)	28(1)
O(2)	2880(3)	8126(2)	6274(1)	28(1)
C(2)	5179(4)	8471(2)	6466(1)	22(1)
O(3)	8637(3)	7375(2)	6858(1)	27(1)
C(3)	6140(3)	7156(2)	6772(1)	20(1)
N(4)	6608(3)	4399(2)	6884(1)	21(1)
C(4)	5790(3)	5592(2)	6541(1)	18(1)
C(5)	7188(4)	5411(2)	6080(1)	24(1)
C(6)	7036(3)	3814(2)	5872(1)	21(1)
C(7)	5092(4)	3365(2)	5599(1)	23(1)
C(8)	4911(3)	1898(2)	5411(1)	22(1)
C(9)	6740(4)	849(2)	5494(1)	20(1)
C(10)	8724(3)	1266(2)	5765(1)	23(1)
C(11)	8850(3)	2740(2)	5948(1)	22(1)
O(12)	6778(3)	-632(1)	5327(1)	26(1)
C(13)	4624(4)	-1184(2)	5117(1)	34(1)

Table 3: *Bond lengths for* ***152***

Bond	Length Å
O(1)-C(1)	1.432(2)
C(1)-C(2)	1.516(2)
O(2)-C(2)	1.424(2)
C(2)-C(3)	1.534(2)
O(3)-C(3)	1.425(2)
C(3)-C(4)	1.525(2)
N(4)-C(4)	1.498(2)
C(4)-C(5)	1.535(2)
C(5)-C(6)	1.514(2)
C(6)-C(7)	1.388(3)
C(6)-C(11)	1.394(3)
C(7)-C(8)	1.389(3)
C(8)-C(9)	1.390(3)
C(9)-O(12)	1.375(2)
C(9)-C(10)	1.395(3)
C(10)-C(11)	1.388(2)
O(12)-C(13)	1.424(3)

Table 4: *Bond angles for* ***152***

Bond	Angle (degrees)
O(1)-C(1)-C(2)	108.87(16)
O(2)-C(2)-C(1)	111.71(16)
O(2)-C(2)-C(3)	111.97(15)
C(1)-C(2)-C(3)	110.93(15)
O(3)-C(3)-C(4)	108.56(14)
O(3)-C(3)-C(2)	109.77(15)
C(4)-C(3)-C(2)	112.25(14)
N(4)-C(4)-C(3)	107.44(13)
N(4)-C(4)-C(5)	109.47(14)
C(3)-C(4)-C(5)	113.31(14)
C(6)-C(5)-C(4)	113.64(14)
C(7)-C(6)-C(11)	117.68(16)
C(7)-C(6)-C(5)	121.39(17)
C(11)-C(6)-C(5)	120.92(16)
C(6)-C(7)-C(8)	122.09(17)
C(9)-C(8)-C(7)	119.04(17)
O(12)-C(9)-C(8)	124.66(18)
O(12)-C(9)-C(10)	115.07(17)
C(8)-C(9)-C(10)	120.27(16)
C(11)-C(10)-C(9)	119.28(17)
C(10)-C(11)-C(6)	121.63(17)
C(9)-O(12)-C(13)	116.70(16)

Symmetry transformations used to generate equivalent atoms:

Table 5: *Anisotropic displacement parameters ($Å^2 \times 10^3$) for **152**. The anisotropic displacement factor exponent takes the form: $-2\,pi^2\,[\,h^2\,a^{*\wedge}\,U11 + \ldots + 2\,h\,k\,a^*\,b^*\,U12\,]$*

	U11	U22	U33	U23	U13	U12
Cl(1)	25(1)	39(1)	24(1)	-7(1)	1(1)	-10(1)
O(1)	44(1)	28(1)	23(1)	-1(1)	10(1)	8(1)
C(1)	40(1)	21(1)	23(1)	0(1)	7(1)	2(1)
O(2)	31(1)	32(1)	21(1)	2(1)	-2(1)	4(1)
C(2)	30(1)	20(1)	17(1)	1(1)	4(1)	1(1)
O(3)	26(1)	33(1)	22(1)	-7(1)	0(1)	-4(1)
C(3)	22(1)	20(1)	17(1)	-1(1)	2(1)	1(1)
N(4)	25(1)	21(1)	19(1)	1(1)	0(1)	2(1)
C(4)	22(1)	18(1)	15(1)	3(1)	-1(1)	0(1)
C(5)	31(1)	23(1)	17(1)	-3(1)	5(1)	-4(1)
C(6)	24(1)	22(1)	16(1)	-1(1)	3(1)	-3(1)
C(7)	23(1)	22(1)	24(1)	1(1)	0(1)	4(1)
C(8)	23(1)	25(1)	20(1)	1(1)	-3(1)	-2(1)
C(9)	26(1)	21(1)	15(1)	0(1)	3(1)	-1(1)
C(10)	20(1)	27(1)	20(1)	1(1)	1(1)	3(1)
C(11)	21(1)	29(1)	17(1)	-3(1)	0(1)	-1(1)
O(12)	30(1)	21(1)	27(1)	-5(1)	-2(1)	2(1)
C(13)	40(1)	26(1)	35(1)	-7(1)	-7(1)	-4(1)

Table 6: *Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($Å^2 \times 10^3$) for* ***152****.*

	x	y	z	U(eq)
H(1)	2708	10662	7143	48
H(1A)	6784	10190	6860	34
H(1B)	4680	10822	6527	34
H(2)	1867	8068	6492	41
H(2A)	6314	8600	6197	27
H(3)	8834	7697	7133	40
H(3A)	5270	7164	7079	23
H(4A)	8240	4399	6899	32
H(4B)	6083	3459	6790	32
H(4C)	5997	4615	7173	32
H(4)	4042	5438	6476	22
H(5A)	6559	6157	5848	28
H(5B)	8895	5664	6137	28
H(7)	3846	4084	5539	28
H(8)	3556	1617	5227	27
H(10)	9973	549	5823	27
H(11)	10207	3025	6131	27
H(13A)	3301	-1102	5342	51
H(13B)	4834	-2260	5026	51
H(13C)	4254	-569	4838	51

Abbreviations

Ac	acetyl
aq.	aqueous
Ar	aryl
atm.	atmosphere
Boc	*tert*-butoxycarbonyl
Bn	benzyl
Bu	butyl
Cbz	benzyloxcarbonyl
CDI	*N,N*-carbonyldiimidazole
DIBAL-H	diisobutylaluminium hydride
DMF	*N,N*-dimethylformamide
DMAP	4-dimethylaminopyridine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
de	diastereomeric excess
ee	enatiomeric excess
Et	ethyl
Ether	diethyl ether
FAB	fast atom bombardment
HMDS	hexamethyldisilazide
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrum
Hz	hertz
IR	infra red

M	mol dm^{-3}
MAP	mitogen-activated protein
Me	methyl
mRNA	messenger ribonucleic acid
Ms	methanesulfonyl
MOM	methoxymethyl
NMR	nuclear magnetic resonance
Ph	phenyl
PMB	*para*-methoxybenzyl
Pr	propyl
ppm	parts per million
Rt	retention time for HPLC
SAR	structure activity relationship
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
Tf	trifluoromethane sulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIBS	triisopropylbenzene sulfonyl
t.l.c.	thin layer chromatography
TMS	trimethylsilyl
tRNA	transfer ribonucleic acid
Ts	*para*-toluene sulfonyl

ORGANIC LETTERS

2002
Vol. 4, No. 2
265−267

An Aldol-Based Approach to the Synthesis of the Antibiotic Anisomycin

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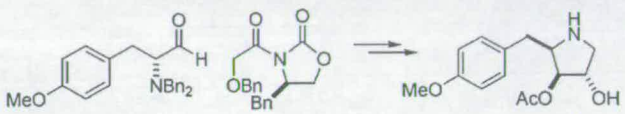
Received November 8, 2001

ABSTRACT



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A new approach to the synthesis of the antibiotic anisomycin is reported that relies upon a key aldol disconnection. The glycolate aldol coupling proceeds in 75% yield and with >95% diastereoselectivity, which allows the 13-step synthesis to proceed in 35% overall yield.

Anisomycin **1** was first isolated from the fermentation broths of *Streptomyces griseolus* and *Streptomyces roseochromogenes* by Sobin and Tanner in 1954.[1] Renewed interest in this antibiotic[2] has arisen from reports of high antitumor activity in vitro, with IC_{50} values in the nanomolar range,[3] and recent studies that have shown that anisomycin may be used in a synergistic fashion with a cyclin-dependent protein kinase inhibitor to kill carcinoma cells.[4] Anisomycin has found widespread use as a tool in molecular biology, where it has been shown to inhibit protein synthesis[5] and to activate JNK and p38 kinases.[6]

Anisomycin has attracted much synthetic interest over the past 30 years, with over 20 syntheses of the antibiotic or its biosynthetic precursor deacetylanisomycin being reported in the literature.[7] However, many of these syntheses have suffered from a series of protection and deprotection steps in the later stages, and the need for an efficient synthesis still remains.

We were attracted to the synthesis of anisomycin **1** following our successful synthesis of the iminosugar DAB-1[8] utilizing a highly diastereoselective *syn* glycolate aldol reaction with a serine-derived α-dibenzylamino aldehyde.[9] We envisaged that a similar approach (Figure 1), using a

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10.1021/ol017016u CCC: $22.00
Published on Web 12/22/2001

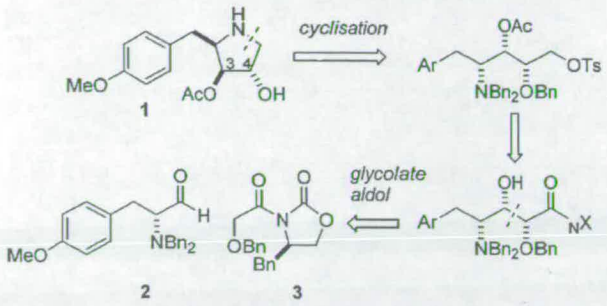
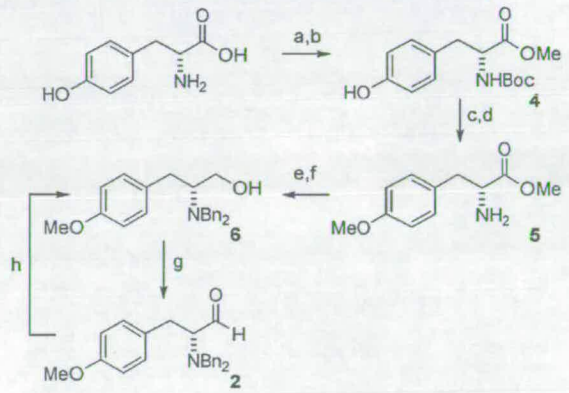


Figure 1. Retrosynthetic analysis of anisomycin (**1**).

tyrosine derived aldehyde **2** and the glycolate derivative of Evans oxazolidinone **3**, would allow a highly efficient synthesis of anisomycin.

D-Tyrosine was readily converted to its methyl ester hydrochloride salt, using in situ generation of the required acid (Scheme 1). Direct benzylation of the amino functionality was found to be impossible in the presence of the phenol; however, the amino functionality could be selectively protected to give the Boc-protected methyl ester **4** in excellent yield. The free phenol was then alkylated with methyl iodide in the presence of potassium carbonate,[10] and treatment with trifluoroacetic acid gave the primary amine **5**. Subsequent *N,N*-dibenzylation ($BnBr$, K_2CO_3) and reduction of the ester with lithium borohydride also proceeded in excellent yield to give the key precursor, amino alcohol **6**.

Scheme 1. Synthesis of Tyrosine-Derived Aldehyde **2**[a]



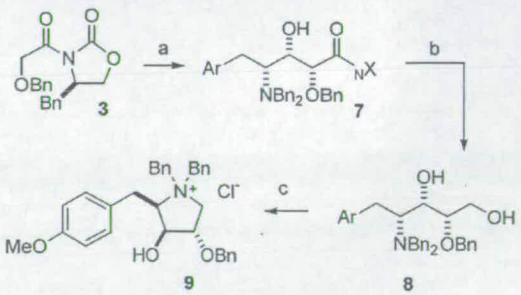
[a] Conditions: (a) AcCl, MeOH (100%); (b) Boc_2O, $NaHCO_3$, EtOH (99%); (c) MeI, K_2CO_3, DMF (97%); (d) TFA, CH_2Cl_2 (100%); (e) BnBr, K_2CO_3, MeCN (95%); (f) $LiBH_4$, MeOH (87%); (g) Swern (100%); (h) DIBAL-H, toluene (90%).

The desired tyrosine-derived aldehyde **2** was obtained via Swern oxidation. We and others[9] have found this two-step reduction/oxidation protocol to be the most efficient for the generation of aldehydes of this type.

The optical purity of alcohol **6** was confirmed by chiral HPLC using a Chiracel OD-H column (solvent; 10% propan-2-ol in hexane). Reassuringly, when compared with traces for the racemic alcohol, this showed that there was no appreciable racemization of alcohol **6** (material >98% ee). Alcohol **6** was found to be a convenient point in the synthesis to store gram quantities of material as a result of its observed stability. Samples of aldehyde **2** (synthesized and isolated using standard Swern procedures) were treated with DIBAL-H to regenerate alcohol **6**. Again the alcohol was confirmed by chiral HPLC to be >98% ee, suggesting that minimal racemization had occurred during the oxidation of **6** to **2**. Thus aldehyde **2** could be produced with high optical purity in seven steps and 80% overall yield from D-tyrosine.

The *syn* glycolate aldol reaction employed in the synthesis of DAB-1[8] was found to be the result of "matched" stereoselectivity of the two components. On the basis of this precedent, it was anticipated that formation of the C(3)–C(4) stereochemistry observed in **1** would require a "mismatched" aldol reaction. In this reaction the stereochemical induction from the oxazolidinone component would be required to outweigh that imposed by the α-chiral aldehyde.[11] The glycolate derivative of Evans oxazolidinone **3** was prepared as described in the literature from benzyloxyacetyl chloride and (4*R*)-benzyloxazolidin-2-one.[12] Formation of the *Z*-boron enolate (Bu_2BOTf, Et_3N, CH_2Cl_2) and reaction with aldehyde **2** gave the desired *syn* aldol adduct **7** in good yield (75%) and as the major diastereomer (>95% de, Scheme 2).[13]

Scheme 2. Glycolate Aldol Coupling[a]



[a] Conditions: (a) (i) Et_3N, Bu_2BOTf, CH_2Cl_2; (ii) **2** (75%); (b) $LiBH_4$, MeOH (80%); (c) (i) TsCl, DMAP, CH_2Cl_2; (ii) Dowex Cl^- (85%).

Aldol adduct **7** was readily reduced to the diol **8** using $LiBH_4$.[14] Selective tosylation of the primary alcohol in the presence of TsCl/DMAP resulted in the formation of the

(9) For a recent review on the role of α-dibenzylamino aldehydes in synthesis, see: Reetz, M. T. *Chem. Rev.* **1999**, *99*, 1121.

(10) The use of K_2CO_3 was shown to be of crucial importance in maintaining the stereochemical integrity of the α-amino ester.

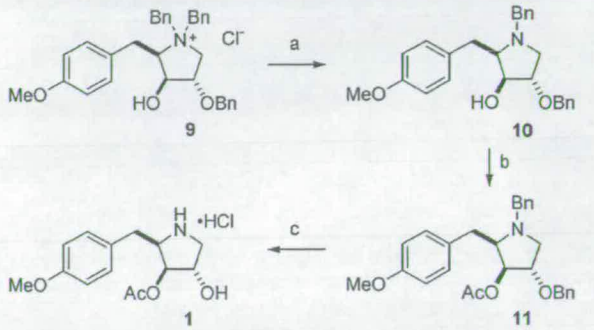
(11) A full analysis of the results of this and other related glycolate aldol reactions will be reported in a separate communication.

(12) Fuhry, M. A. M.; Holmes, A. B.; Marshall, D. R. *J. Chem Soc., Perkin Trans. 1* **1993**, 2743.

pyrrolidinium tosylate salt,[15] with no evidence of ditosylated material being formed. Conversion to the chloride salt **9** was achieved using Dowex resin treated with 1% HCl. Counterion exchange was confirmed by the absence of the diagnostic tosylate peaks [observed at $\delta = 2.29$ (s), 7.83 (d, J 8.2 Hz) and 7.10 (d, J 8.2 Hz)] in the ^{1}H NMR spectrum.

Completion of the synthesis of anisomycin **1** was achieved most efficiently via partial deprotection of the quaternary salt (Scheme 3). When the salt **9** was subjected to hydrogenation under basic conditions for 10 min or less, the benzyl-protected pyrrolidine **10** could be isolated in high yield (94%). Acetylation of this benzylated material was found to be more efficient than direct acylation of the chloride salt. This is presumed to be due to the increased solubility of the substrate, which also greatly enhances the ease of isolation of the product material. Final debenzylation was found to require "fresh" palladium hydroxide for complete deprotection to take place. Difficulties associated with partial salt formation during Celite filtration of the deprotected pyrrolidine were obviated by using acidic conditions provided by the addition of 2 equiv of a 1 M solution of HCl in ether to the reaction mixture to generate the pyrrolidine hydrochloride salt. This allowed analytically pure anisomycin **1** to be isolated as its hydrochloride salt in quantitative yield.

Scheme 3. Synthesis of Anisomycin (**1**)[a]



[a] Conditions: (a) Pd/C (cat.), K_2CO_3, H_2, MeOH, 10 min (94%); (b) Ac_2O, Et_3N, DMAP, CH_2Cl_2 (92%); (c) $Pd(OH)_2$ (cat.), H_2, HCl, MeOH (100%).

In summary, an extremely rapid synthesis of anisomycin **1** has been achieved in 13 steps with a 35% overall yield. The synthesis relies upon a key glycolate aldol coupling, which has been shown to proceed in high yield and with excellent diastereoselectivity. Of note, this synthesis does not require extensive protecting group swaps in the final steps. Rather, the synthesis relies upon a stepwise deprotection of the benzyl protecting groups that were introduced at an early stage in the synthesis. The efficiency of the synthesis of the differentially protected aldehyde **2** (prepared in 80% overall yield from D-tyrosine) will allow future access to a wide range of analogues using this synthetic route.

Acknowledgment. We thank the BBSRC for financial support of this work (grant 99/A1/B/05153 to E.M.R.).

Supporting Information Available: Experimental procedures for the synthesis of compounds **7**−**10** and spectral data for compounds **11** and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) A synthetic route involving selective mono-*N*-debenzylation of the aldol adduct **7**, cyclization to a protected pyrrolidin-2-one, and then reduction to the protected pyrrolidine **10** was not pursued, as in practice it was found to be most convenient to carry out the aldol coupling and reduction to alcohol **8** without purification of the intermediate aldol adduct **7**.

(14) The Evans oxazolidinone was also recovered in reasonable yield (60%).

(15) Similar mesylate-induced cyclizations have been observed in the synthesis of *N*-(3-pyrrolodinylmethyl)benzamides: Thomas, C.; Hübner, H.; Gmeiner, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 841.